

PIPECOLIC ACID DERIVATIVES OF PROLINE THREONINE  
AMIDES USEFUL FOR THE TREATMENT OF RHEUMATOID  
ARTHRITIS

5

Field of the Invention

Non-peptidic compounds are described for treatment of autoimmune diseases. Of particular interest are  
10 pipecolic acid derivatives compounds incorporating a characterizing segment condensed from pipecolic acid, aspartic acid, proline and threonine, or derivatives thereof, which compounds are useful to treat rheumatoid arthritis.

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Background of the Invention

Rheumatoid arthritis (RA) is an autoimmune disease  
20 characterized by chronic inflammation of the synovium. This disease is triggered by an immune response generated via the molecular recognition of the T-cell receptor on CD4-positive T cells with a complex of disease-inducing peptides bound to Human Leukocyte Antigen (HLA) class II  
25 molecules.

Rheumatoid arthritis (RA) is associated with the expression of certain HLA class II molecules, particularly the DR4-dw4, as well as DR1 and DR4-dw14.  
30 It is known that blockade of the interaction between a given class II molecule, peptide ligand, and T cell receptor inhibits specific T cell responses both in vitro and in vivo. It is further known that blockade of the above interaction in animal models of autoimmunity  
35 prevents or ameliorates autoimmune disease. Inhibitor compounds which block the binding of disease-inducing peptides to an RA-associated HLA molecule, but which will not interfere with a patient's ability to generate other class II-restricted immune responses, constitutes a

selective immunosuppressive anti-RA therapy. Compounds which compete with disease-inducing endogenous peptides for binding to RA-associated HLA molecules and may thereby inhibit disease.

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Other therapeutic strategies which are directed at the T cell, such as total lymphoid irradiation, thoracic duct drainage, cyclosporin A, anti-CD4 monoclonal antibody, and other monoclonal antibodies directed at T cell determinants, result in some cases in clinical improvement of rheumatoid arthritis, but these therapies are also associated with side effects. For instance, conventional general immunosuppressives increase the risk of opportunistic infections and cancer.

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PCT App Pub #WO 93/05011, published 18 March 1993, describes a series of oligopeptides, typically comprised of ten or more amino acids, which bind to HLA Class II molecules for indication against autoimmune diseases.

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These larger peptides incorporating proteogenic amino acids can have poor oral bioavailability and poor plasma stability, as well as difficulty in penetrating cell membranes for proper tissue distribution.

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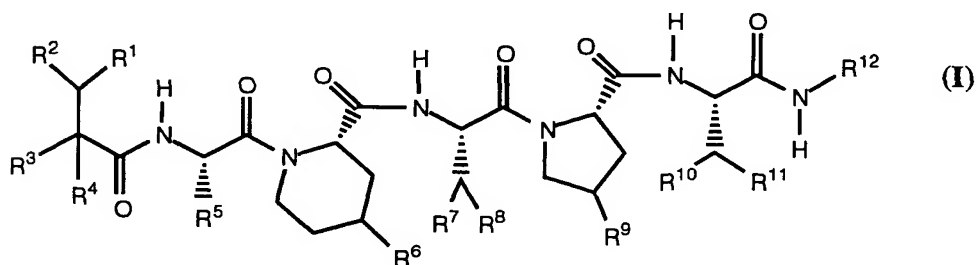
A series of heptapeptides, such as recently reported to bind to HLA Class II molecules, similarly comprising natural, mammalian amino acids, unblocked at the C and N termini, are likely to be unstable in vivo and to possess poor bioavailability [Hammer et al, PNAS, 91, 4456-4460

30

(1994)] .

Description of the Invention

Treatment of an autoimmune or inflammatory disease, or a hypersensitive reaction of the acute or delayed type, or an allergic reaction or asthmatic disorder, or treatment of dermatitis, arthritis, meningitis, granulomas, vasculitis or septic shock, may be accomplished by preventing or suppressing an immune response in a treatment subject by use of a pipecolic acid derivative of a proline threonine amide selected from a family of compounds of general Formula I:



- 15 wherein R<sup>1</sup> is selected from alkyl, phenyl, cycloalkyl rings having four to ten ring-member carbon atoms, bicycloalkyl fused ring systems having seven to nine ring-member carbon atoms, heteroaryl, heteroarylalkyl, benzo-fused-heteroaryl and benzo-fused-heteroarylalkyl
- 20 wherein said heteroaryl moiety or fragment is a 5- or 6-ring-member fully-unsaturated ring system having one hetero atom as a ring member, said hetero atom selected from oxygen, nitrogen and sulfur atoms, and wherein any of said heteroaryl, heteroarylalkyl, benzo-fused-
- 25 heteroaryl and benzo-fused-heteroarylalkyl may be attached to the nucleus of Formula I as an R<sup>1</sup> substituent through a bond formed at any said ring-member atom or any atom of the alkyl portion of said R<sup>1</sup> substituent where said bond is capable of forming a stable compound;
- 30 wherein R<sup>2</sup> is selected from hydrido, lower alkyl, cyclohexyl and phenyl;

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wherein  $R^3$  is selected from hydrido, hydroxy, lower alkyl, phenyl, acetyl(Lys)NH-, acetyl(Tyr)NH-, acetyl(Thr)NH-, acetylamino, propionylamino and benzyloxycarbonylamino;

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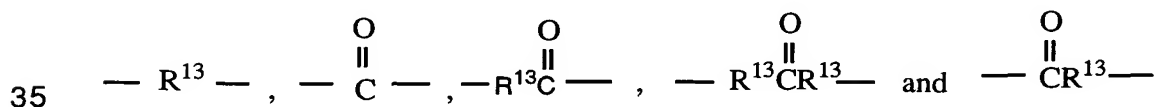
wherein  $R^4$  is selected from hydrido, lower alkyl and phenyl;

10 wherein  $R^5$  is selected from hydrido, lower alkyl, phenyl, benzyl, hydroxyphenyl, hydroxybenzyl, aminoalkyl, mono-alkyl-substituted-aminoalkyl and radicals provided by B-Het-A;

15 wherein Het is selected from heteroaryl moieties consisting of monocyclic and fused bicyclic ring systems having a total of five to fourteen ring members and with one to six ring members being selected from hetero atoms provided by oxygen, nitrogen and sulfur atoms, wherein said monocyclic ring system and at least one ring system  
20 of said fused bicyclic ring system is fully unsaturated, and

wherein Het is further selected from heterocyclic moieties consisting of monocyclic and fused polycyclic  
25 ring systems having a total of four to twelve ring members and with one to six ring members selected from hetero atoms provided by oxygen, nitrogen and sulfur atoms, wherein said monocyclic ring-system and at least one ring system of said fused polycyclic ring system is  
30 fully saturated or partially unsaturated,

wherein A is a single covalent bond or is a divalent radical selected from



wherein  $R^{13}$  is lower alkyl;

wherein B is one or more substituents attached at a substitutable position on Het of Het-A, said substituent selected from hydrido, hydroxy, alkyl, cycloalkyl, cycloalkylalkyl, alkoxy, hydroxyalkyl, alkoxyalkyl, carboxy, alkenyl, alkynyl, halo, haloalkyl, oxo, cyano, benzyl and phenyl;

wherein R<sup>6</sup> is selected from hydrido, lower alkyl, hydroxy, alkoxy, alkoxyalkyl, carboxyalkyl, alkoxycarbonyl, alkoxycarbonyloxy, aminoalkyl, mono-alkyl-substituted-aminoalkyl, amido and amidoalkyl;

wherein R<sup>7</sup> is selected from carboxyl, lower alkyl, amido and methylthiomethyl;

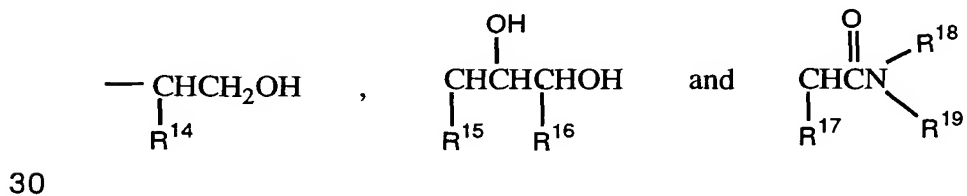
wherein R<sup>8</sup> is selected from hydrido, methyl and ethyl;

wherein R<sup>9</sup> is selected from hydrido, lower alkyl, alkoxy and phenyl;

wherein R<sup>10</sup> is hydrido or hydroxy;

wherein R<sup>11</sup> is hydrido or methyl;

wherein R<sup>12</sup> is selected from lower alkyl, phenyl, phenylalkyl, cycloalkyl, cycloalkylalkyl,



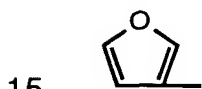
wherein each of R<sup>14</sup> through R<sup>17</sup> is independently selected from hydrido, hydroxy, alkyl, hydroxyalkyl, alkoxy, alkoxyalkyl, cycloalkyl, cycloalkylalkyl, halo, haloalkyl, cyano, benzyl and phenyl;

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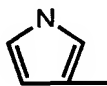
wherein each of R<sup>18</sup> and R<sup>19</sup> is independently selected from hydrido, alkyl, cycloalkyl, cycloalkylalkyl, benzyl and phenyl;

- 5 or a pharmaceutically-acceptable amide, ester or salt thereof.

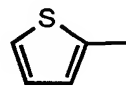
10 Examples of heteroaryl 5- or 6-ring member monocyclic ring systems and benzo-fused bicyclic ring systems, having one hetero atom as a ring member selected from oxygen, nitrogen and sulfur atoms, and which ring systems are fully unsaturated, i.e. "aromatic" in character, in at least one ring, are as follow:



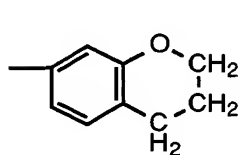
furyl



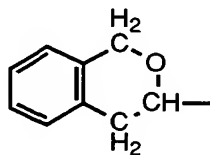
pyrrolyl



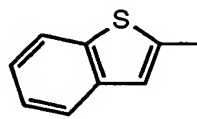
thienyl



chromanyl

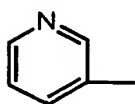


isochromanyl

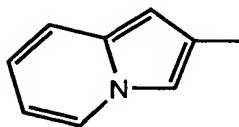


benzothienyl

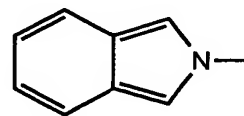
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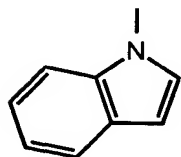
pyridyl



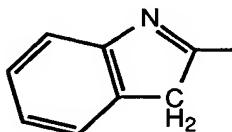
indoliziny



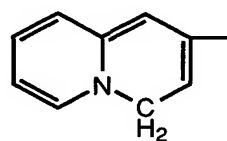
isoindolyl



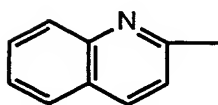
25. indolyl



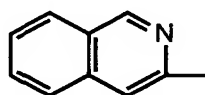
3H-indolyl



quinoliziny

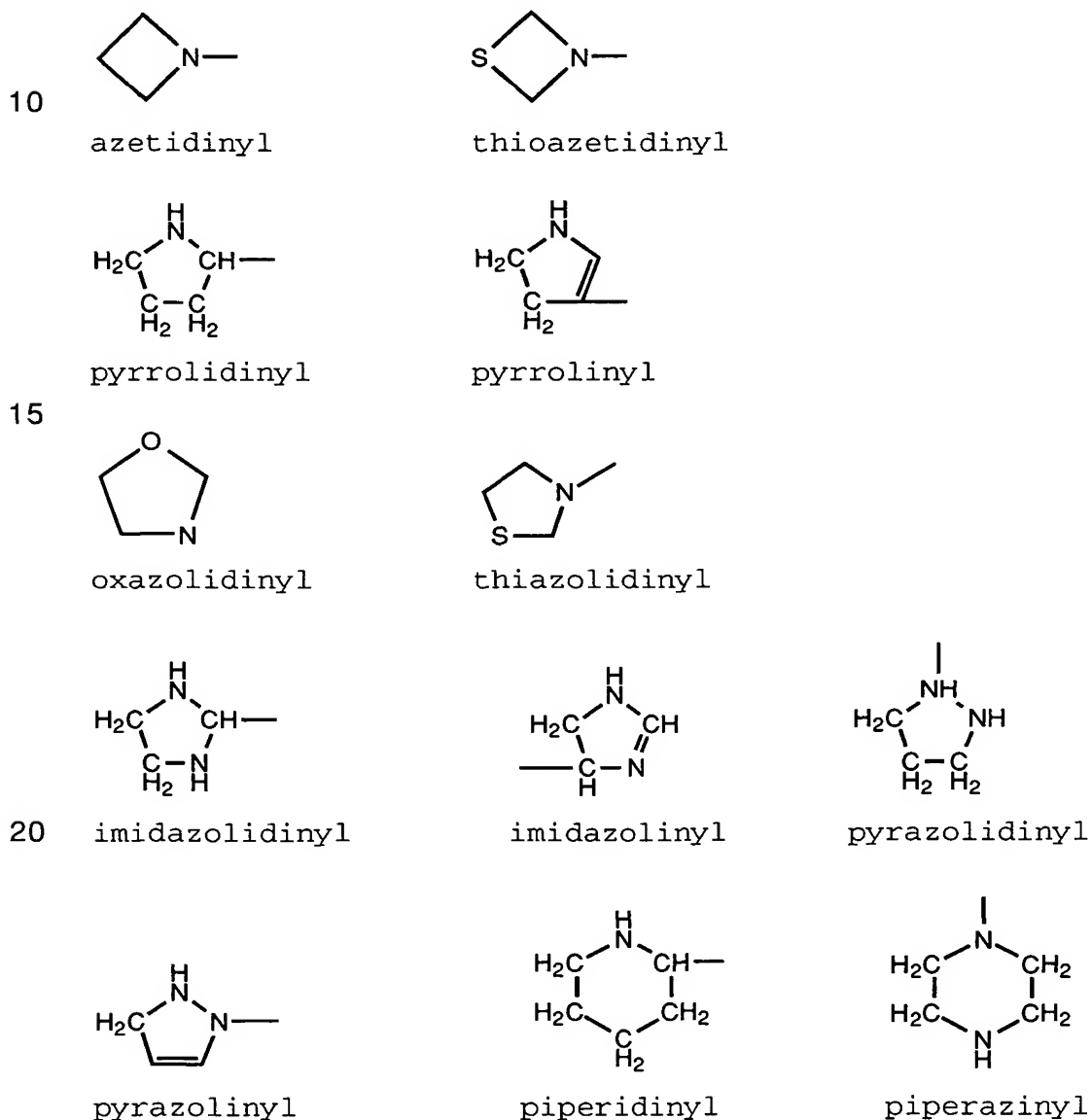


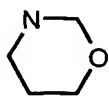
quinolyl



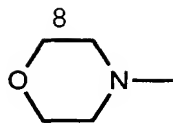
isoquinolyl

Examples of heterocyclic-type monocyclic or polycyclic ring systems having four to twelve ring members with one to six hetero atoms as ring members, said hetero atoms selected from oxygen, nitrogen and sulfur atoms, and wherein at least one of such ring systems is fully saturated or partially unsaturated, and wherein the polycyclic ring system may be composed of a benzo-fused ring, are as follow:

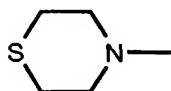




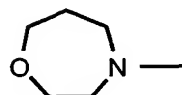
1,3-morpholino



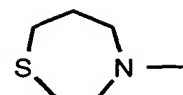
1,4-morpholino



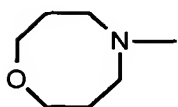
5 1,4-thiomorpholino



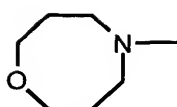
oxazopinyll



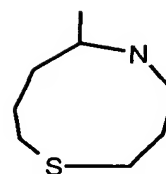
thiazopinyll



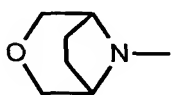
oxazocinyll



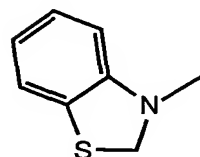
thiazocinyll



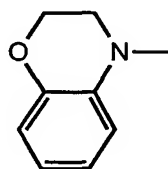
azoninyll



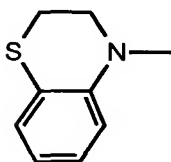
10 oxazabicyclo



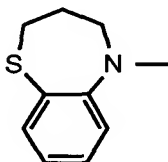
benzo-fused-thiazolidinyll



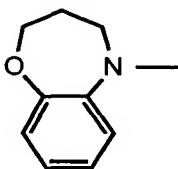
benzo-fused-morpholino



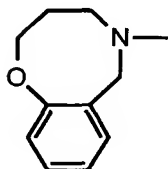
benzo-fused thiomorpholinyll



benzo-fused-thiazopinyll

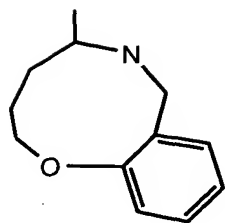


20 benzo-fused oxazopinyll

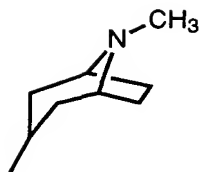


benzo-fused-oxazocinyll

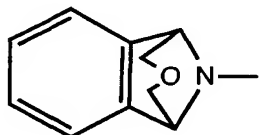




benzo-fused-oxazoninyl

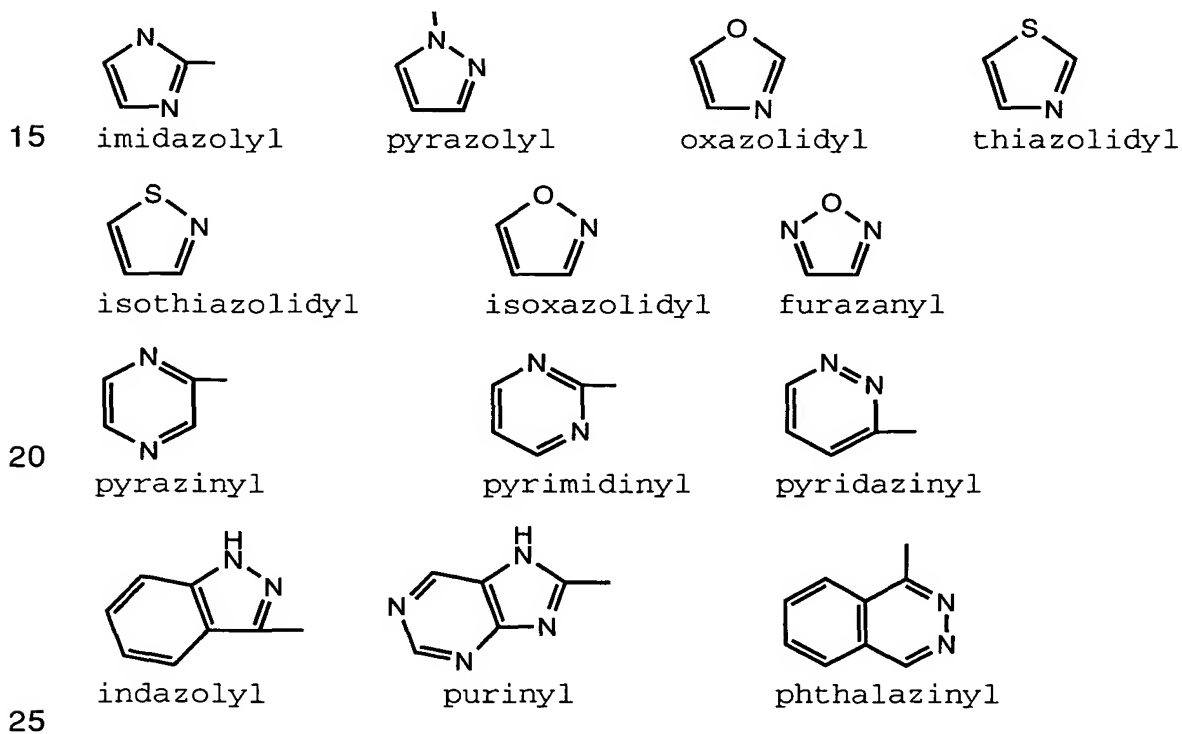


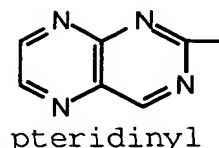
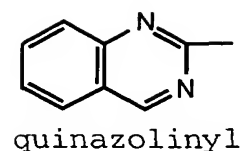
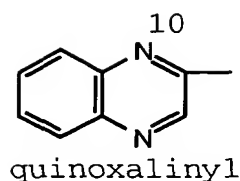
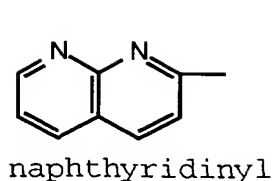
tropanyl



5 benzo-fused-oxazobicyclo

Examples of heteroaryl monocyclic and bicyclic ring systems and benzo-fused polycyclic ring systems, having one to six hetero atoms as ring members selected from oxygen, nitrogen and sulfur atoms, and which ring systems are fully unsaturated, i.e. "aromatic" in character, in at least one of the ring system, are as follow:





A preferred family of compounds consists of compounds of Formula I:

- 10 wherein R<sup>1</sup> is selected from cyclopentyl, cyclohexyl, cycloheptyl, norbornanyl, phenyl, furyl, pyrrolyl, thienyl, chromanyl, isochromanyl, benzothienyl, pyridyl, indoliziny1, isoindoly1, indoly1, 3H-indoly1, quinoliziny1, quinoly1, isoquinoly1, azetidiny1,
- 15 thioazetidiny1, pyrrolidiny1, pyrroliny1, oxazolidiny1, thiazolidiny1, imidazolidiny1, imidazolinyl, pyrazolidiny1, pyrazolinyl, piperidiny1, piperaziny1, 1,3-morpholino, 1,4-morpholino, 1,4-thiomorpholino, azepiny1, oxazopiny1, thiazopiny1, oxazociny1,
- 20 thiazociny1, azoniny1, oxazabicyclo, benzo-fused-oxazolidiny1, benzo-fused-thiazolidiny1, benzo-fused-morpholino, benzo-fused thiomorpholinyl, benzo-fused-thiazopiny1, benzo-fused oxazopiny1, benzo-fused-oxazociny1, benzo-fused-oxazoniny1, tropany1 and benzo-
- 25 fused-oxazobicyclo;

wherein R<sup>2</sup> is selected from hydrido, methyl, ethyl, propyl, cyclohexyl and phenyl;

- 30 wherein R<sup>3</sup> is selected from hydrido, hydroxy, methyl, ethyl, phenyl, acetyl(Lys)NH-, acetyl(Tyr)NH-, acetyl(Thr)NH-, acetylamino, propionylamino and benzyloxycarbonylamino;

- 35 wherein R<sup>4</sup> is hydrido or methyl;

wherein R<sup>5</sup> is selected from hydrido, n-propyl, isopropyl, n-butyl, isobutyl, phenyl, benzyl, hydroxyphenyl, hydroxybenzyl, aminopropyl, aminobutyl and radicals

5 provided by B-Het-R<sup>13</sup> and B-Het-CR<sup>13</sup>;

wherein Het is selected from furyl, pyrrolyl, thienyl, chromanyl, isochromanyl, benzothienyl, pyridyl, indoliziny, isoindolyl, indolyl, 3H-indolyl, 10 quinoliziny, quinolyl, isoquinolyl, imidazolyl, pyrazolyl, oxazolidyl, thiazolidyl, isothiazolidyl, isoxazolidyl, furazanyl, pyrazinyl, pyrimidinyl, pyridazinyl, indazolyl, purinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, 15 pteridinyl, thieno-furanyl, furopyranyl, pyrido-oxazinyl, pyrazolo-oxazolyl, imidazo-thiazolyl, pyrazino-pyridazinyl, imidazo-thiazolyl, oxothiolo-pyrrolyl, imidazo-triazinyl, benzoxazinyl, azetidiny, thioazetidiny, pyrrolidinyl, pyrrolinyl, oxazolidinyl, 20 thiazolidinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperidinyl, piperazinyl, 1,3-morpholino, 1,4-morpholino, 1,4-thiomorpholino, azepinyl, oxazopinyl, thiazopinyl, oxazocinyl, thiazocinyl, azoninyl, oxazabicyclo, benzo-fused- 25 oxazolidinyl, benzo-fused-thiazolidinyl, benzo-fused-morpholino, benzo-fused thiomorpholinyl, benzo-fused-thiazopinyl, benzo-fused oxazopinyl, benzo-fused-oxazocinyl, benzo-fused-oxazoninyl, tropanyl and benzo-fused-oxazobicyclo;

30 wherein R<sup>13</sup> is lower alkyl;

wherein B is one or more substituents attached at a substitutable position on Het, said substituent selected 35 from hydrido, hydroxy, alkyl, cycloalkyl, cycloalkylalkyl, alkoxy, hydroxyalkyl, alkoxyalkyl, oxo, benzyl and phenyl;

wherein  $R^6$  is selected from hydrido, lower alkyl, hydroxy, methoxy carboxyalkyl, alkoxycarbonyl, alkoxycarbonyloxy, aminoalkyl, mono-alkyl-substituted-  
 5 aminoalkyl, amido and amidoalkyl;

wherein  $R^7$  is selected from carboxyl, lower alkyl, amido and methylthiomethyl;

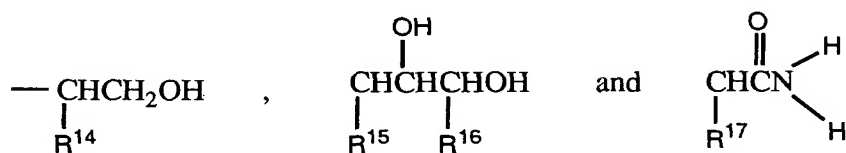
10 wherein  $R^8$  is hydrido or methyl;

wherein  $R^9$  is selected from hydrido, lower alkyl, methoxy and phenyl;

15 wherein  $R^{10}$  is hydrido or hydroxy;

wherein  $R^{11}$  is hydrido or methyl;

20 wherein  $R^{12}$  is selected from lower alkyl, phenyl, benzyl, phenylethyl, cyclohexylethyl,



25 wherein each of  $R^{14}$  through  $R^{17}$  is independently selected from hydrido, hydroxy and alkyl;

or a pharmaceutically-acceptable amide, ester or salt thereof.

30

A more preferred family of compounds consists of compounds of Formula I:

wherein  $R^1$  is selected from cyclopentyl, cyclohexyl, cycloheptyl, norbornanyl, phenyl, azetidiny, thioazetidiny, pyrrolidiny, pyrroliny,  
 35

13

oxazolidinyl, thiazolidinyl, imidazolidinyl,  
 imidazoliny, pyrazolidinyl, pyrazoliny,  
 piperidinyl, piperazinyl 1,3-morpholino, 1,4-morpholino,  
 1,4-thiomorpholino, azepiny, oxazopiny, thiazopiny,  
 5 oxazociny, thiazociny, azoniny, oxazabicyclo and  
 tropanyl;

wherein R<sup>2</sup> is selected from hydrido, methyl, ethyl,  
 propyl, acetyl(Lys)NH-, acetyl(Tyr)NH-, acetyl(Thr)NH-,  
 10 cyclohexyl and phenyl;

wherein R<sup>3</sup> is selected from hydrido, hydroxy, methyl,  
 ethyl, phenyl, acetylamino, propionylamino and  
 benzyloxycarbonylamino;

15 wherein R<sup>4</sup> is hydrido or methyl;

wherein R<sup>5</sup> is selected from hydrido, n-propyl, isopropyl,  
 n-butyl, isobutyl, aminopropyl, aminobutyl, phenyl,  
 20 hydroxyphenyl, benzyl, hydroxybenzyl and radicals

O  
||

provided by B-Het-CR<sup>13</sup>;

wherein Het is selected from azetidiny, pyridiny,  
 isoindoly, oxazolyl, isoxazolyl, indoly, quinoly,  
 25 isoquinoly, azetidiny, thioazetidiny, pyrrolidinyl,  
 pyrroliny, oxazolidinyl, thiazolidinyl, imidazolyl,  
 imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazoliny,  
 piperidinyl, piperazinyl,  
 1,3-morpholino, 1,4-morpholino, 1,4-thiomorpholino,  
 30 azepiny, oxazopiny, thiazopiny, oxazociny,  
 thiazociny, azoniny, oxazabicyclo and tropanyl;

wherein R<sup>13</sup> is selected from methyl, ethyl and propyl;

35 wherein B is one or more substituents attached at a  
 substitutable position on Het, said substituent selected

from hydrido, hydroxy, methyl, ethyl, propyl, oxo, benzyl<sup>14</sup> and phenyl;

wherein R<sup>6</sup> is selected from hydrido, methyl, hydroxy,  
5 methoxy, phenyl, alkoxycarbonyl, alkoxycarbonyloxy, aminoalkyl, mono-amido and amidoalkyl;

wherein R<sup>7</sup> is selected from carboxyl, n-propyl, n-butyl,  
10 amido and methylthiomethyl;

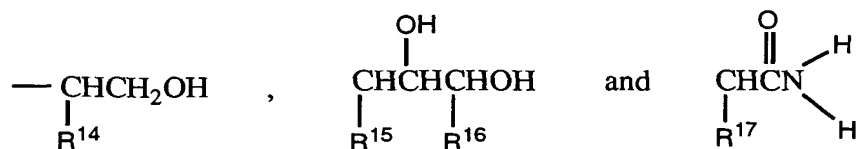
wherein R<sup>8</sup> is hydrido or methyl;

wherein R<sup>9</sup> is selected from hydrido, lower alkyl, methoxy  
15 and phenyl;

wherein R<sup>10</sup> is hydroxy;

wherein R<sup>11</sup> is hydrido or methyl;

20 wherein R<sup>12</sup> is selected from lower alkyl, phenyl, phenylethyl, cyclohexylethyl,



25 wherein each of R<sup>14</sup> through R<sup>17</sup> is independently selected from hydrido, hydroxy, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, benzyl and phenyl;

30 or a pharmaceutically-acceptable amide, ester or salt thereof.

A highly preferred family of compounds consists of compounds of Formula I:

wherein R<sup>1</sup> is phenyl or cyclohexyl; wherein R<sup>2</sup> is hydrido or methyl; wherein R<sup>3</sup> is selected from hydrido, hydroxy, acetyl(Lys)NH-, acetyl(Tyr)NH-, acetyl(Thr)NH-, acetylamino, propionylamino and benzyloxycarbonylamino; wherein R<sup>4</sup> is hydrido; wherein R<sup>5</sup> is selected from isopropyl, isobutyl, n-propyl, n-butyl, aminopropyl, aminobutyl, phenyl, benzyl, para-hydroxyphenyl, para-hydroxybenzyl, imidazolcarbonylethyl, imidazolcarbonylpropyl, pyrrolidinylcarbonylethyl, pyrrolidinylcarbonylpropyl, azetidylcarbonylethyl, azetidylcarbonylpropyl, morpholinocarbonylethyl, morpholinocarbonylpropyl, piperazinocarbonylethyl, piperazinocarbonylpropyl, pyridinylcarbonylethyl, pyridinylcarbonylpropyl, oxazolylcarbonylethyl, oxazolylcarbonylpropyl, isoxazolylcarbonylethyl, isoxazolylcarbonylpropyl, azepinylcarbonylethyl and azepinylcarbonylpropyl; wherein R<sup>6</sup> is selected from hydrido, methyl, hydroxy, methoxy, phenyl and aminocarbonyl; wherein R<sup>7</sup> is carboxyl or methylthiomethyl; wherein R<sup>8</sup> is hydrido; wherein R<sup>9</sup> is selected from hydrido, hydroxy, methyl, methoxy and phenyl; wherein R<sup>10</sup> is hydroxy; wherein R<sup>11</sup> is methyl; wherein R<sup>12</sup> is selected from methyl, ethyl, propyl, butyl, isobutyl, -CH(iBu)CH<sub>2</sub>OH and -CH(iBu)CONH<sub>2</sub>; or a pharmaceutically-acceptable amide, ester or salt thereof.

The term "hydrido" denotes a single hydrogen atom (H). This hydrido group may be attached, for example, to an oxygen atom to form a hydroxyl group; or, as another example, one hydrido group may be attached to

a carbon atom to form a  $\begin{array}{c} \diagup \\ \text{CH} \\ \diagdown \end{array}$  group; or, as another

example, two hydrido atoms may be attached to a carbon atom to form a -CH<sub>2</sub>- group. Where the term "alkyl" is used, either alone or within other terms such as "haloalkyl" and "hydroxyalkyl", the term "alkyl" embraces

linear or branched radicals having one to about twenty carbon atoms or, preferably, one to about twelve carbon atoms. More preferred alkyl radicals are "lower alkyl" radicals having one to about ten carbon atoms. Most preferred are lower alkyl radicals having one to about five carbon atoms. The term "cycloalkyl" embraces cyclic radicals having three to about ten ring carbon atoms, preferably three to about six carbon atoms, such as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The term "haloalkyl" embraces radicals wherein any one or more of the alkyl carbon atoms is substituted with one or more halo groups, preferably selected from bromo, chloro and fluoro. Specifically embraced by the term "haloalkyl" are monohaloalkyl, dihaloalkyl and polyhaloalkyl groups. A monohaloalkyl group, for example, may have either a bromo, a chloro, or a fluoro atom within the group. Dihalalkyl and polyhaloalkyl groups may be substituted with two or more of the same halo groups, or may have a combination of different halo groups. A dihaloalkyl group, for example, may have two fluoro atoms, such as difluoromethyl and difluorobutyl groups, or two chloro atoms, such as a dichloromethyl group, or one fluoro atom and one chloro atom, such as a fluoro-chloromethyl group. Examples of a polyhaloalkyl are trifluoromethyl, 1,1-difluoroethyl, 2,2,2-trifluoroethyl, perfluoroethyl and 2,2,3,3-tetrafluoropropyl groups. The term "difluoroalkyl" embraces alkyl groups having two fluoro atoms substituted on any one or two of the alkyl group carbon atoms. The terms "alkylol" and "hydroxyalkyl" embrace linear or branched alkyl groups having one to about ten carbon atoms any one of which may be substituted with one or more hydroxyl groups. The term "alkenyl" embraces linear or branched radicals having two to about twenty carbon atoms, preferably three to about ten carbon atoms, and containing at least one carbon-carbon double bond, which carbon-carbon double bond may have either cis or trans geometry within the alkenyl moiety. The term "alkynyl"



embraces linear or branched radicals having two to about twenty carbon atoms, preferably two to about ten carbon atoms, and containing at least one carbon-carbon triple bond. The term "cycloalkenyl" embraces cyclic radicals  
5 having three to about ten ring carbon atoms including one or more double bonds involving adjacent ring carbons. The terms "alkoxy" and "alkoxyalkyl" embrace linear or branched oxy-containing radicals each having alkyl portions of one to about ten carbon atoms, such as  
10 methoxy group. The term "alkoxyalkyl" also embraces alkyl radicals having two or more alkoxy groups attached to the alkyl radical, that is, to form monoalkoxyalkyl and dialkoxyalkyl groups. The "alkoxy" or "alkoxyalkyl" radicals may be further substituted with one or more  
15 halo atoms, such as fluoro, chloro or bromo, to provide haloalkoxy or haloalkoxyalkyl groups. The term "alkylthio" embraces radicals containing a linear or branched alkyl group, of one to about ten carbon atoms attached to a divalent sulfur atom, such as a methylthio  
20 group. Preferred aryl groups are those consisting of one, two, or three benzene rings. The term "aryl" embraces aromatic radicals such as phenyl, naphthyl and biphenyl. The term "aralkyl" embraces aryl-substituted alkyl radicals such as benzyl, diphenylmethyl, triphenyl-  
25 methyl, phenyl-ethyl, phenylbutyl and diphenylethyl. The terms "benzyl" and "phenylmethyl" are interchangeable. The terms "phenalkyl" and "phenylalkyl" are interchangeable. An example of "phenalkyl" is "phenethyl" which is interchangeable with "phenylethyl".  
30 The terms "alkylaryl", "alkoxyaryl" and "haloaryl" denote, respectively, the substitution of one or more "alkyl", "alkoxy" and "halo" groups, respectively, substituted on an "aryl" nucleus, such as a phenyl moiety, which is then attached to the structure of  
35 Formula I or Formula II. The terms "aryloxy" and "arylthio" denote radicals respectively, provided by aryl groups having an oxygen or sulfur atom through which the radical is attached to a nucleus, examples of which are

phenoxy and phenylthio. The terms "sulfinyl" and "sulfonyl", whether used alone or linked to other terms, denotes, respectively, divalent radicals SO and SO<sub>2</sub>. The term "aralkoxy", alone or within another term, embraces  
5 an aryl group attached to an alkoxy group to form, for example, benzyloxy. The term "acyl" whether used alone, or within a term such as acyloxy, denotes a radical provided by the residue after removal of hydroxyl from an organic acid, examples of such radical being acetyl and  
10 benzoyl. "Lower alkanoyl" is an example of a more preferred sub-class of acyl. The term "amido" denotes a radical consisting of nitrogen atom attached to a carbonyl group, which radical may be further substituted in the manner described herein. The amido radical can be  
15 attached to the nucleus of a compound of Formula I or Formula II through the carbonyl moiety or through the nitrogen atom of the amido radical. The term "monoalkylaminocarbonyl" is interchangeable with "N-alkylamido". The term "dialkylaminocarbonyl" is  
20 interchangeable with "N,N-dialkylamido". The term "alkenylalkyl" denotes a radical having a double-bond unsaturation site between two carbons, and which radical may consist of only two carbons or may be further substituted with alkyl groups which may optionally  
25 contain additional double-bond unsaturation. The term "heteroaryl", where not otherwise defined before, embraces aromatic ring systems containing one or two hetero atoms selected from oxygen, nitrogen and sulfur in a ring system having five or six ring members, examples  
30 of which are thienyl, furanyl, pyridinyl, thiazolyl, pyrimidyl and isoxazolyl. Such heteroaryl may be attached as a substituent through a carbon atom of the heteroaryl ring system, or may be attached through a carbon atom of a moiety substituted on a heteroaryl ring-  
35 member carbon atom, for example, through the methylene substituent of imidazolemethyl moiety. Also, such heteroaryl may be attached through a ring nitrogen atom as long as aromaticity of the heteroaryl moiety is

preserved after attachment. For any of the foregoing defined radicals, preferred radicals are those containing from one to about ten carbon atoms.

- 5                    Specific examples of alkyl groups are methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, isopentyl, methylbutyl, dimethylbutyl and neopentyl. Typical alkenyl and alkynyl groups may have one unsaturated bond, such as an allyl
- 10 group, or may have a plurality of unsaturated bonds, with such plurality of bonds either adjacent, such as allene-type structures, or in conjugation, or separated by several saturated carbons.
- 15                    Also included in the combination of the invention are the isomeric forms of the above-described compounds of Formula I, including diastereoisomers, regioisomers and the pharmaceutically-acceptable salts thereof. The term "pharmaceutically-acceptable salts"
- 20 embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically-acceptable. Suitable
- 25 pharmaceutically-acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids may be
- 30 selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, example of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic,
- 35 anthranilic, p-hydroxybenzoic, salicylic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, pantothenic, benzenesulfonic, toluenesulfonic, sulfanilic, mesylic,

cyclohexylaminosulfonic, stearic, algenic,  
β-hydroxybutyric, malonic, galactaric and galacturonic  
acid. Suitable pharmaceutically-acceptable base addition  
salts include metallic salts made from aluminium,  
5 calcium, lithium, magnesium, potassium, sodium and zinc  
or organic salts made from N,N'-dibenzylethylenediamine,  
chloroprocaine, choline, diethanolamine, ethylenediamine,  
meglumine (N-methylgluca-mine) and procaine. All of  
these salts may be prepared by conventional means from  
10 the corresponding compound by reacting, for example, the  
appropriate acid or base with such compound.

Nomenclature used to define the amino acids  
used to make compounds of Formula I is that specified by  
15 the IUPAC [published in European Journal of Biochemistry,  
138, 9-37 (1984)], wherein conventional representation of  
the peptides stipulates that in a peptide sequence the  
amino group appears to the left and the carboxyl group to  
the right. When the amino acid has enantiomeric forms,  
20 it is the L form of the amino acid which is represented  
unless otherwise stated. In the amino acid structural  
formulas, each residue is generally represented by a  
single or 3-letter designation, corresponding to the  
trivial name of the amino acid in accordance with the  
25 following list:

	<u>TRIVIAL NAME</u>	<sup>21</sup> <u>SYMBOL</u>	<u>ONE-LETTER SYMBOL</u>
	Alanine	Ala	A
	Arginine	Arg	R
5	Asparagine	Asn	N
	Aspartic Acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic Acid	Glu	E
10	Glycine	Gly	G
	Histidine	His	H
	Homocysteine	Hcy	-
	Isoleucine	Ile	I
	Leucine	Leu	L
15	Lysine	Lys	K
	Methionine	Met	M
	Norvaline	Nva	-
	Penicillamine	Pen	-
	Phenylalanine	Phe	F
20	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
25	Valine	Val	V
	Unspecified Amino Acid	Xaa	X

Another name for norvaline is n-propylglycine. The group <sup>125</sup>I-Tyr indicates a radioactive mono-iodinated tyrosine residue.

35 CBA = 3-cyclohexylbutyric acid  
 CPA = 3-cyclohexylpropionic acid  
 Ac = acetyl  
 OBn = benzyloxy  
 Pec = pipercolic acid  
 Z = benzyloxycarbonyl

Compounds of the present invention function as HLA "groove blockers", referring to the peptide binding pocket or "groove" present in the HLA, particularly the PR4-dw4, as well as DR1 and DR4-dw14. Because the  
5 compounds of the present invention are targeted to specific subsets of Class II molecules, greater specificity and better side-effect profiles are gained. Compounds of the present invention find utility in the treatment of other autoimmune diseases, including  
10 rheumatoid arthritis.

Thus, compounds of Formula I would be useful in suppressing immune response in a human or animal subject susceptible to or afflicted with an autoimmune disease or  
15 inflammatory disease. Examples of such treatable disease are systemic lupus erythematosus, multiple sclerosis, myasthenia gravis, thyroiditis, Grave's disease, autoimmune hemolytic anemia, autoimmune hemolytic anemia, autoimmune thrombocytopenia purpura, mixed connective  
20 tissue disease, idiopathic Addison's disease, Sjögren's syndrome, insulin dependent diabetes mellitus, rheumatoid arthritis, psoriasis, glomerulonephritis, inflammatory bowel disease and Crohn's Disease.

25 Compounds of Formula I would also be useful in suppressing immune response in a human or animal subject susceptible to or afflicted with an allergy, such as an asthmatic condition or reaction, urticaria or with airway hypersensitivity.

30

Compounds of Formula I would also be useful in suppressing immune response in a human or animal subject afflicted with or susceptible to septic shock.

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Compounds of Formula I would also be useful in preventing or suppressing acute or delayed-type hypersensitivity responses or conditions resulting from or associated with hypersensitivity responses such as

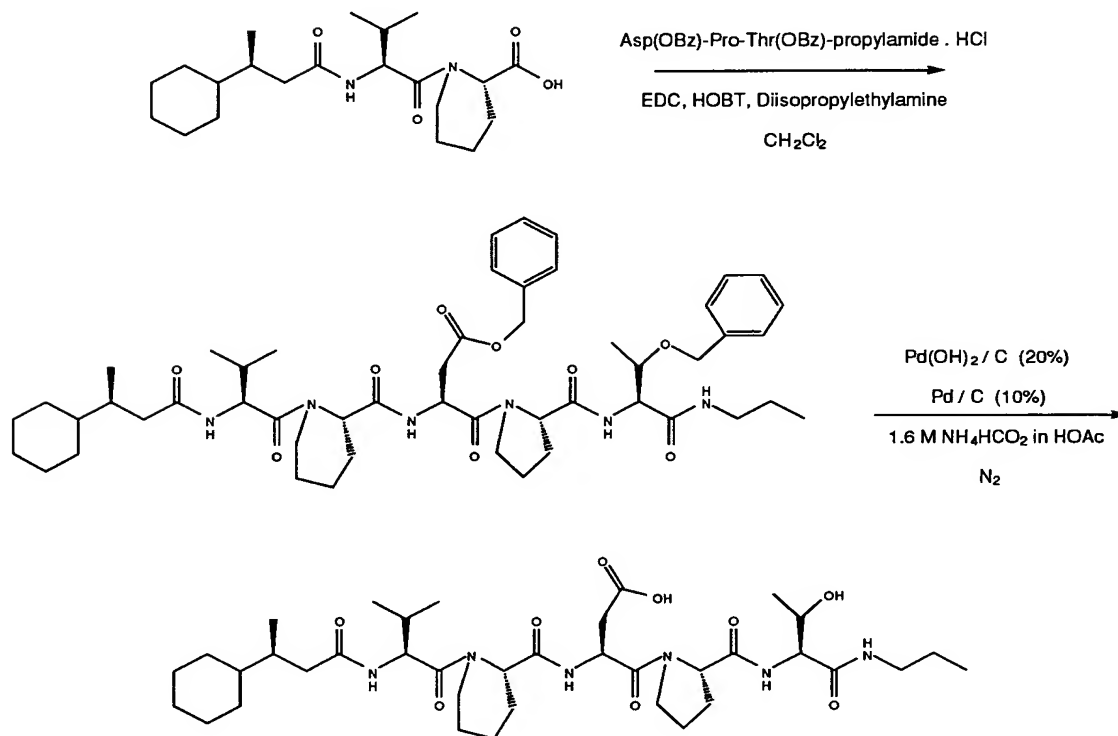
contact dermatitis, hemolytic anemias, antibody-induced thrombocytopenia, Goodpasture's syndrome, hypersensitivity, pneumonitis, glomerulonephritis, granulomas, thyroiditis, encephelomyelitis and  
5 meningitis.

Of particular interest is use of a compound of Formula I to treat rheumatoid arthritis.

10 Compounds of Formula I would also be useful in adjunct therapy involving, typically, coadministration of a second immunosuppressive compound of Formula I or coadministration of an immunosuppressive agent of a different class of compounds. Such coadministration may  
15 provide a synergistic result between a compound of Formula I and an agent selected from one or more other classes of immunosuppressants. Such synergistic result allows for a lower dosage of another immunosuppressant agent having a toxic effect, such as a cyclosporin agent  
20 or steroid agent, such as cortisone and cortisol. Thus, use of a compound of Formula I in such synergistic combination allows utilization of the immunosuppressant benefits of a cyclosporin agent or steroid agent without, or with less of, the deleterious side effects of such  
25 agent. Examples of other immunosuppressant agents include a cyclosporin compound, Fujisawa FK-506 (macrolide lactone) compound, rapamycin, a glucocorticoid, an antiproliferative agent, a monoclonal antibody such as an anti-CD3 (anti-T cell receptor  
30 antibody), anti-CD5/CD7, anti-CD4 agent, an anti-IL-2 receptor (anti-cytokine receptor antibody) agent, an anti-IL-2 (anti-cytokine antibody), Nippon NKT-01 (15-deoxyspergualin) and Syntex RS-61443.

# Description of Synthetic Methods for Preparation of Compounds of the Invention

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## Synthesis

10

The compounds of Formula I can be prepared by coupling individual protected amino acid building blocks by standard solution or solid phase methods. The coupling reagents may be selected from those amide-

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forming reagents known in the art, such as carbodiimides, mixed carbonic anhydrides and active esters, but are not limited to these methods. The following Steps and

Examples constitute specific exemplification of methods to prepare starting materials, intermediates and final

20

compounds of Formula I. Those skilled in the art will readily understand that known variations of the conditions and processes of the following preparative procedures can be used to prepare the compounds of the



Steps and Examples. All temperatures expressed are in degrees Centigrade.

### Step 1

#### 5 Pro-Thr(OBn)-*n*-propylamide hydrochloride

Commercially available Boc-L-Thr(OBn)-OH (5g) was mixed with 1-hydroxybenzotriazole (2.62g), diisopropylethylamine (2.08g) in methylene chloride (14mL) and cooled to 0°C. To this was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, followed by *n*-propylamine (1.14g) 2 minutes later. After allowing the reaction mixture to stir at 0 for 5 hours, the organic solution was washed sequentially with water, 15 1N hydrochloric acid, 5% sodium bicarbonate, then dried (magnesium sulfate), filtered and evaporated to obtain an oily residue. This residue was taken up in 4M hydrogen chloride in dioxane solution (50mL) and allowed to stand at room temperature for 30 minutes. The solvent was then 20 evaporated and the product triturated with ether. The resulting solid was collected a filter plate. A portion of this material (1.5g) was then mixed with Boc-L-Pro-OSu (1.5g) in methylene chloride (20mL), cooled to 0°C, and diisopropylethylamine (675mg) was added. After allowing 25 the reaction mixture to stir at 0°C for 5 hours, the organic solution was washed sequentially with water, 1N hydrochloric acid, 5% sodium bicarbonate, then dried (magnesium sulfate), filtered and evaporated to obtain an oily residue. This residue was taken up in 4 M hydrogen 30 chloride in dioxane solution (50mL) and allowed to stand at room temperature for 30 minutes. The solvent was then evaporated and the product triturated with ether. The resulting solid was collected a filter plate to yield the title compound (1.45g). <sup>1</sup>H NMR (400MHz): consistent with 35 proposed structure.

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**Step 2**Asp(OBn)-Pro-Thr(OBn)-*n*-propylamide hydrochloride

Commercially available Boc-L-Asp(OBn)-OH (1g) was  
5 mixed with 1-hydroxybenzotriazole (500mg), the title  
compound from Step 1 (1g) and diisopropylethylamine  
(736mg) in methylene chloride (20mL), and the solution  
was cooled to 0°C. To this was added 1-(3-  
10 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride  
(710mg) in one portion. After allowing the reaction  
mixture to stir at 0°C for 5 hours, the organic solution  
was washed sequentially with water, 1 N hydrochloric  
acid, 5% sodium bicarbonate, then dried (magnesium  
15 sulfate), filtered and evaporated to obtain an oily  
residue. This residue was taken up in 4 M hydrogen  
chloride in dioxane solution (50mL) and allowed to stand  
at room temperature for 30 minutes. The solvent was then  
evaporated and the product triturated with ether. The  
20 resulting white solid was collected a filter plate to  
yield the title compound. <sup>1</sup>H NMR (400MHz): consistent  
with proposed structure.

**Step 3**Pec-Asp(OBn)-Pro-Thr(OBn)-*n*-propylamide hydrochloride

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Commercially available Boc-L-pipecolic acid (Boc-  
Pec, 100mg) was mixed with 1-hydroxybenzotriazole (71mg),  
the title compound from Step 2 (259mg) and  
diisopropylethylamine (113mg) in methylene chloride  
30 (2mL), and the solution was cooled to 0°C. To this was  
added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide  
hydrochloride (100mg) in one portion. After allowing the  
reaction mixture to stir at 0°C for 5 hours, the organic  
solution was washed sequentially with water, 1N  
35 hydrochloric acid, 5% sodium bicarbonate, then dried  
(magnesium sulfate), filtered and evaporated. The  
residue was taken up in 4M hydrogen chloride in dioxane  
solution (50mL) and allowed to stand at room temperature

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for 30 minutes. The solvent was then evaporated and the product triturated with ether. The resulting white solid was collected a filter plate to yield the title compound (223mg).  $^1\text{H}$  NMR (400MHz): consistent with proposed structure.

**Step 4**

Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-*n*-propylamide hydrochloride

10

The same procedure as described in Step 3 was used to couple Boc-L-Lys(Z)-OH to the title compound of Step 3, yielding the title compound as a white solid (1.12g).

**Step 5**

Val-Pec-OMe hydrochloride

15

Boc-Val-OH was dissolved in  $\text{CH}_2\text{Cl}_2$ , and cooled to  $0^\circ\text{C}$  via ice water bath. The reaction solution was treated with 1.2 eq of 1-hydroxybenzotriazole, 2 eq of diisopropylethylamine, and 1.5 eq of Pec-OMe·HCl. After all solids were dissolved, the reaction was treated with 1.2 eq of 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide · HCl. The reaction flask was stoppered, sealed with parafilm, and placed in the refrigerator overnight. The  $\text{CH}_2\text{Cl}_2$  was evaporated and replaced with EA. The organic solution was washed with water, 1.5 M HCl, and 5%  $\text{NaHCO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , and thoroughly evaporated to a clear, colorless oil. The entire sample was then dissolved in 4.0M HCl in dioxane, stirred for one hour at rt, and thoroughly evaporated to yield Val-Pec-OMe·HCl as a white foam.

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**Step 6***(S)*-3-cyclohexylbutyryl-Val-Pec-OH*((S)*-CBA-Val-Pec-OH )

5           Commercially available *(S)*-3-phenylbutyric acid  
treated with rhodium-on-carbon in methanol at 60 psi and  
60°C for 3.2 hours to yield *(S)*-3-cyclohexylbutyric acid.  
This acid was then converted to the acid chloride by  
treatment with 1 equivalent of oxalyl chloride and a  
10       catalytic amount of DMF, in toluene for 90 minutes. 250  
mg of resulting *(S)*-3-cyclohexylbutyryl chloride was  
dissolved in 10 mL ethyl acetate, and treated with 1.5  
equivalents of the dipeptide, Val-Pec-OMe hydrochloride,  
followed by 3 equivalents of sodium bicarbonate dissolved  
15       in 2 mL of water. The reaction mixture stirred  
vigorously for 2 hours. The organic solution was then  
washed with 1.5 M HCl, dried over sodium sulfate, and  
evaporated to obtain a clear, colorless oil. This  
product was then treated with 3 equivalents of KOH  
20       dissolved in water and tetrahydrofuran and the reaction  
stirred vigorously for 12 hours. The tetrahydrofuran was  
evaporated, and the acid product was precipitated by  
acidifying the aqueous solution with *conc.* HCl. The  
mixture was extracted with ethyl acetate, the organic  
25       layer dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to yield the  
title compound as a white foam.

**Step 7***s*-CBA-Val-Pec-Asp(OBn)-Pro-Thr(OBn)-propylamide

30

100 mg of the title compound from Step 6 (*(S)*-CBA-  
Val-Pec-OH) was dissolved in 3 mL of dichloromethane, and  
cooled to 0°C via ice water bath. The clear, colorless  
reaction solution was then treated with 36 mg of 1-  
35       hydroxybenzotriazole, 94 mL of diisopropylethylamine, and  
170 mg of Asp(OBn)-Pro-Thr(OBn)-propylamide  
hydrochloride, while stirring. After all solids had  
dissolved, the reaction solution was treated with 51 mg

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- of 1-(3-dimethylaminopropyl)-3-ethylcarbo- dimide hydrochloride. The solution stirred at 0° C for 1 hour. The reaction flask was then stoppered, sealed with parafilm, and placed in the refrigerator overnight. The
- 5 dichloromethane solvent was then evaporated and replaced with Ethyl Acetate. The organic solution was washed with water, 1.5M HCl, and 5%Na<sub>2</sub>CO<sub>3</sub>, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated thoroughly. The entire sample was flash chromatographed eluting with 5% MeOH/CHCl<sub>3</sub>.
- 10 The title compound (s-CBA-Val-Pec-Asp(OBz)-Pro-Thr(OBz)-propylamide) was successfully isolated (62 mg) as a white crystalline solid.

#### Step 8

- 15 (S)-3-Cyclohexylbutyroyl chloride

- (S)-3-phenylbutyric acid was hydrogenated using Rh/C in MeOH at 60 psi and 60°C for 3.2 hours. The reduced product was then converted to the title acid chloride by
- 20 treating with 1 eq of oxalyl chloride, and a catalytic amount of DMF, in toluene for 90 minutes, followed by evaporation of the volatile materials.

#### Step 9

- 25 Thr(O-benzyl)-allyl ester hydrochloride

- To a room temperature solution of commercially available Boc-Threonine(O-benzyl)-OH (8.00g, 25.0 mmol) and cesium carbonate (11.32g, 34.7 mmol) in dry DMF (15.5
- 30 mL) under a N<sub>2</sub> atmosphere, was added by syringe allyl bromide (3.01 mL, 34.7 mmol). After stirring overnight the mixture was concentrated, diluted with H<sub>2</sub>O (75mL) and extracted with ethyl acetate (3 x 25 mL). The combined organic layers were washed with saturated NaHCO<sub>3</sub> solution
- 35 (2 x 25 mL), H<sub>2</sub>O(2 x 25 mL), and brine (25 mL), then dried with MgSO<sub>4</sub>. The filtrate was concentrated to a clear, colorless oil (8.76g). <sup>1</sup>H NMR (400MHz) data was consistent for the Boc compound. The oil was dissolved

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into 4 M HCl in 1,4-dioxane (100 mL) and stirred at room temperature for 30 minutes. The reaction was concentrated to a clear, yellow oil, triturated several times with Et<sub>2</sub>O, and concentrated to a oil (7.40g, 109% crude yield).

5 <sup>1</sup>H NMR (400MHz): consistent with proposed structure.

#### Step 10

Pro-Thr(O-benzyl)-allyl ester hydrochloride

10 To a 0°C solution of the title compound from Step 9 (6.80g, 23.8 mmol) and commercially available Boc-proline-Osu (6.81g, 21.8mmol) in CH<sub>2</sub>Cl<sub>2</sub> (70mL) under a N<sub>2</sub> atmosphere, was added diisopropylethylamine (4.32 mL, 24.8 mmol). After 2h at 0°C the solution was washed with water  
15 (20 mL), 1.0 N KHSO<sub>4</sub> solution (20 mL), saturated NaHCO<sub>3</sub> (2 x 20 mL), H<sub>2</sub>O (2 X 20 mL) and brine (20 mL). Dried over MgSO<sub>4</sub>. The filtrate was concentrated and purified by medium pressure chromatography (silica gel, 30% ethyl acetate in hexane) to give a white solid (5.66g). The  
20 solid was dissolved into 4 N HCL in 1,4-dioxane (100 mL) at room temperature. After 1 hour the solution was concentrated, triturated with Et<sub>2</sub>O several times, and concentrated to give the title compound as a sticky, white solid (4.61g, 55% yield). <sup>1</sup>H NMR (400MHz): consistent with  
25 proposed structure.

#### Step 11

Asp(O-benzyl)-Pro-Thr(O-benzyl)-allyl ester hydrochloride

30 To a 0°C solution of the tile compound from Step 10 (4.61g, 12.0 mmol), Boc-Asp(O-benzyl)-OH (3.89g, 12.0mmol), 1-hydroxybenzotriazole (4.41g, 32.6 mmol) and 4-methyl morpholine (1.39g, 13.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (65 mL) under a N<sub>2</sub> atmosphere, was added 1-(3-  
35 dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (3.28g, 17.1 mmol). The solution was stirred at 0°C for 1 hour and then room temperature overnight. It was then concentrated and

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redissolved into CH<sub>2</sub>Cl<sub>2</sub> (100 mL), and washed with 1.0 N KHSO<sub>4</sub> solv (2 x 25 mL), saturated NaHCO<sub>3</sub> solv (2 x 25 mL), H<sub>2</sub>O (2 x 25 mL), and brine (25mL). Dried over MgSO<sub>4</sub>. The filtrate was concentrated and purified by medium pressure chromatography (silica gel, 30% ethyl acetate in hexane) to give a clear, colorless oil (6.88g). The oil was dissolved into 4N HCl in dioxane at rt. After 30 minutes the solution was concentrated to a white solid, triturated with Et<sub>2</sub>O several times, and dried in a 50°C vacuum oven to give the title compound as a white solid (4.65g, 66% yield). <sup>1</sup>H NMR (400MHz): consistent with proposed structure. Anal. Calcd for C<sub>30</sub>H<sub>38</sub>N<sub>3</sub>O<sub>7</sub>Cl<sub>1</sub>: C, 61.27; H, 6.51; N, 7.15. Found: C, 61.05; H, 6.71; N, 7.14.

15

**Step 12****Lys(Z)-Pec-OMe hydrochloride**

Commercially available Boc-Lys(Z)-OH was dissolved in dimethylformamide (DMF), and cooled to 0°C via ice water bath. The solution was treated with 1.2 equivalents of 1-hydroxybenzotriazole, 2 equivalents of diisopropylethylamine, and 1.5 equivalents of commercially available pipecolic methyl ester hydrochloride (Pec-OMe·HCl). After all solids were dissolved, the reaction was treated with 1.2 equivalents of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. Reaction progress was monitored by TLC. After 5 hours, the DMF was thoroughly evaporated. The oily residue was dissolved in water/ethyl acetate. The organic portion was washed with 1.5 M HCl and 5% NaHCO<sub>3</sub>, dried over MgSO<sub>4</sub>, and filtered. The solution was evaporated to a clear, colorless oil. The entire sample was then dissolved in 4.0 M HCl in dioxane, stirred for one hour at rt, and thoroughly evaporated to yield the title compound, Lys(Z)-Pec-OMe·HCl, as a white foam.

**Step 13***(S)*-CBA-Lys(Z)-Pec-OH

1.0 g of the title compound from Step 8 *(S)*-3-cyclohexylbutyroyl chloride was dissolved in 15 mL ethyl acetate, and treated with 1.5 equivalents (eq) of the title compound from Step 12, Lys(Z)-Pec-OMe·HCl, followed by 3 eq of NaHCO<sub>3</sub> dissolved in 5 mL of water. After 30 minutes, the organic portion was washed with water, 1.5 M HCl and 5% NaHCO<sub>3</sub>. The solution was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to a clear, colorless oil (89.4%). The entire sample was chromatographed by flash column eluting with 5% MeOH/CHCl<sub>3</sub> to remove a baseline impurity. The entire sample was then hydrolyzed using 3 eq of KOH dissolved in water and tetrahydrofuran (THF) as a cosolvent. The reaction stirred vigorously for 5 hours. The THF was evaporated and the aqueous portion was acidified with concentrated HCl. The mixture was extracted with ethyl acetate, dried over Na<sub>2</sub>SO<sub>4</sub>, and the solution evaporated to a clear, colorless oil (72%), which was lyophilized from water to give the title compound as a white solid.

**Step 14***(S)*-CBA-Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-allyl ester

The title compound from Step 13 (300 mg), *(S)*-CBA-Lys(Z)-Pec-OH, was dissolved in 3 mL of CH<sub>2</sub>Cl<sub>2</sub>, and cooled to 0°C via ice bath. The clear, colorless reaction solution was then treated with 76 mg 1-hydroxybenzotriazole, 145 µL diisopropylethylamine, and 365 mg of the title compound from Step 11, Asp(OBz)-Pro-Thr(OBz)-allyl ester·HCl, while stirring. After all solids had dissolved, the reaction solution was treated with 108 mg of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl. After 4.5 hours, the CH<sub>2</sub>Cl<sub>2</sub> was evaporated and replaced with ethyl acetate. The organic solution was washed with water, 1.5 M HCl and 5% NaHCO<sub>3</sub>,



33

dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated to a clear, colorless oil. The entire sample was chromatographed by flash column eluting with 5% MeOH/CHCl<sub>3</sub>. The title compound (407 mg), (*S*)-CBA-Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-allyl ester, was isolated as a clear, colorless oil (67.5% yield).

#### Step 15

(*S*)-CBA-Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-OH

10

2.2 mg of palladium acetate were dissolved in 2 mL dry THF. This clear, light orange solution was then treated with 5 mg of triphenylphosphine. In a separate vial, 138 µL of triethylamine was diluted with 1 mL dry THF and treated with 29 µL of formic acid. This complex was then added to the reaction solution, which promptly turned a dark green color. Finally, the title compound from Step 14 (407 mg), (*S*)-CBA-Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-allyl ester was dissolved in 10 mL dry THF and added to the reaction solution. The atmosphere within the reaction vessel was evacuated and replaced with a steady stream of nitrogen. Reaction progress was monitored by TLC. After 86 hours, the solution was filtered through celite, and the clear, pale yellow filtrate was thoroughly evaporated to a sticky foam. The entire sample was chromatographed on silica gel, eluting with 10% MeOH/CHCl<sub>3</sub> plus 0.5% HOAc. After purification and lyophilization, 208 mg of the title compound, (*S*)-CBA-Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-OH was isolated as a white solid (52.8% yield).

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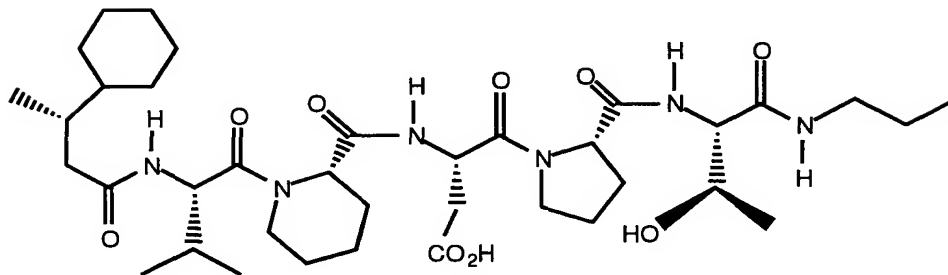
#### Step 16

(*S*)-CBA-Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-leucinol

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60 mg of the title compound from Step 15, (*S*)-CBA-Lys(Z)-Pec-Asp(OBz)-Pro-Thr(OBz)-OH were dissolved in 2 mL of CH<sub>2</sub>Cl<sub>2</sub>, and cooled to 0°C via ice water bath. The clear, colorless reaction solution was treated with 9 mg

34  
of 1-hydroxybenzotriazole, 22  $\mu$ L of  
diisopropylethylamine, and 11  $\mu$ L of commercially  
available leucinol, while stirring. After all solids had  
dissolved, the reaction was treated with 13 mg of 1-(3-  
5 dimethylaminopropyl)-3-ethylcarbodiimide. The reaction  
flask was then stoppered, sealed with parafilm, and  
placed in the refrigerator overnight. The  $\text{CH}_2\text{Cl}_2$  was  
thoroughly evaporated and replaced with ethyl acetate.  
The organic portion was washed with water, 1.5 M HCl, and  
10 5%  $\text{Na}_2\text{CO}_3$ , dried over  $\text{Na}_2\text{SO}_4$ , and evaporated to yield 48  
mg of the title compound, (S)-CBA-Lys(Z)-Pec-Asp(OBz)-  
Pro-Thr(OBz)-leucinol as a white solid (73% yield).

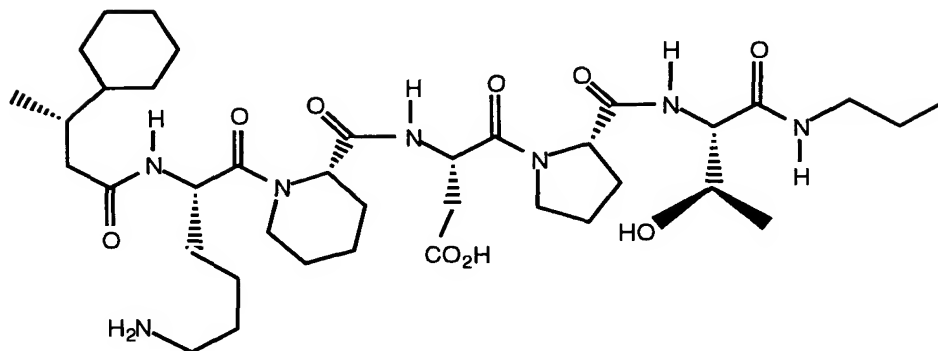
**Example 1***(S)*-CBA-Val-Pec-Asp-Pro-Thr-propylamide

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The title compound from Step 7 (62 mg of *(S)*-CBA-Val-Pec-Asp(OBz)-Pro-Thr(OBz)-propylamide) was mixed with 65 mg of 20% Pd(OH)<sub>2</sub>/C and 62 mg of 10% Pd/C in a 10 mL round bottom flask. This mixture was then treated with 0.5 mL of a 1.6M solution of NH<sub>4</sub>HCO<sub>2</sub> in acetic acid, while stirring. The atmosphere in the reaction vessel was then evacuated and replaced with nitrogen. After 4 hours, the reaction was diluted with methanol and filtered through a celite plug to remove the catalyst. The resulting clear, colorless filtrate was thoroughly evaporated. The oily residue was dissolved in 3 mL distilled water and lyophilized. The title compound, was obtained (46 mg) as a fluffy, white solid (92%).

Analysis by NMR (D<sub>2</sub>O) verified that all protecting groups had been successfully removed. The title compound exhibited one single peak by HPLC. <sup>1</sup>H NMR (400MHz): consistent with proposed structure. Mass spectrum (m/e): 735.

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**Example 2***(S)*-CBA-Lys-Pec-Asp-Pro-Thr-propylamide

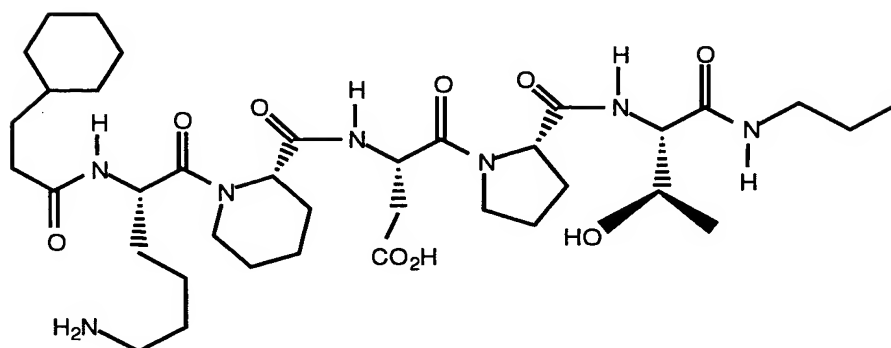
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5                    *(S)*-3-cyclohexylbutyryl chloride (13mg) was added  
 to a two-phase mixture of water, ethyl acetate, the title  
 compound of Step 4 (60mg) and sodium bicarbonate (10mg).  
 10 After vigorously stirring for 30 minutes, the ethyl  
 acetate phase was isolated and washed with 1N  
 hydrochloric acid, then dried (magnesium sulfate) and the  
 solvent evaporated. The residue was mixed with 65 mg of  
 20% Pd(OH)<sub>2</sub>/C and 62 mg of 10% Pd/C in a 10 mL round  
 15 bottom flask. This mixture was then treated with 0.5 mL  
 of a 1.6M solution of NH<sub>4</sub>HCO<sub>2</sub> in acetic acid, while  
 stirring, under nitrogen. After 4 hours, the reaction  
 was diluted with methanol and filtered through a celite  
 plug to remove the catalyst. The resulting clear,  
 20 colorless filtrate was thoroughly evaporated. The oily  
 residue was dissolved in 3 mL distilled water and  
 lyophilized. The title compound was obtained (37 mg) as  
 a fluffy, white solid (as the acetate salt). <sup>1</sup>H NMR  
 (400MHz): consistent with proposed structure. Mass  
 25 spectrum (m/e): 764.

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**Example 3**

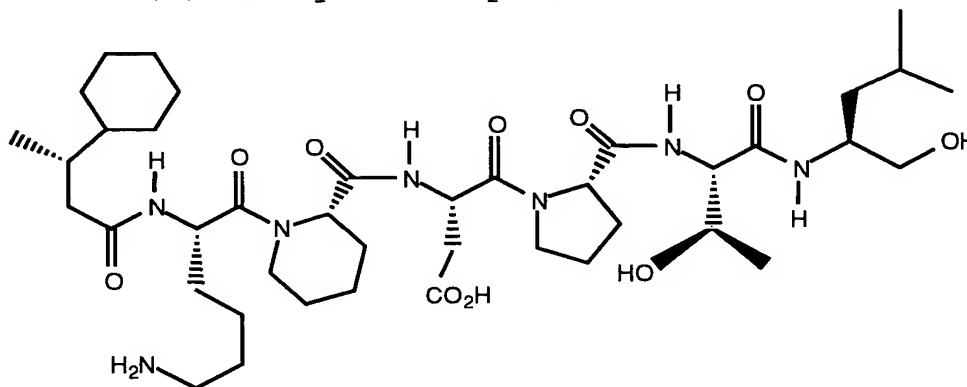
CPA-Lys-Pec-Asp-Pro-Thr-propylamide



5

The same procedure of Example 2 was employed substituting cyclohexylpropionyl chloride in place of (*S*)-3-cyclohexylbutyryl chloride gave the title compound (as the acetate salt). <sup>1</sup>H NMR (400MHz): consistent with proposed structure. Mass spectrum (m/e): 750.

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**Example 4**(*S*)-CBA-Lys-Pec-Asp-Pro-Thr-leucinol

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40 mg of the title compound of Step 16, (*S*)-CBA-Lys(Z)-Pec-Asp(OBn)-Pro-Thr(OBn)-leucinol, were combined with 45 mg of 20% Pd(OH)<sub>2</sub>/C and 40 mg of 10% Pd/C in a 10 mL round bottom flask. This mixture was then treated with 0.5 mL of a 1.6 M solution of NH<sub>4</sub>HCO<sub>2</sub> in HOAc, while stirring. The atmosphere in the reaction vessel was evacuated and replaced with a steady stream of N<sub>2</sub>. After 4 hours, the reaction was diluted with MeOH and filtered

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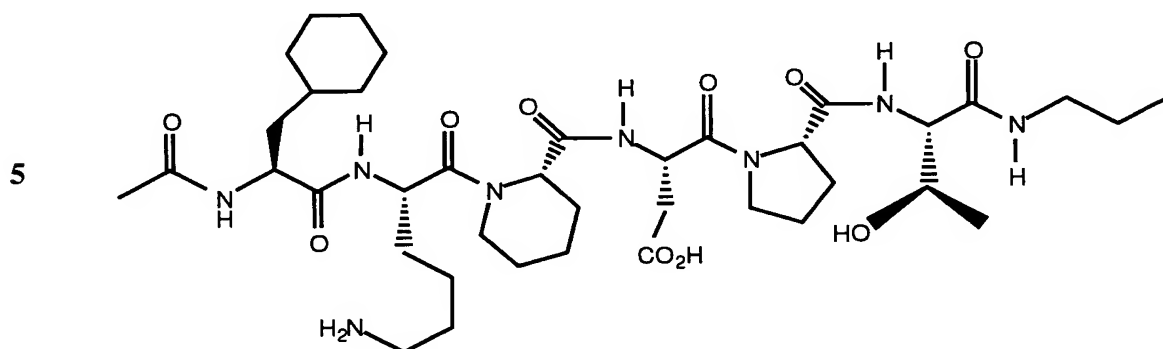
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through a celite plug to remove all catalyst. The clear, colorless filtrate was thoroughly evaporated. The oily residue was dissolved in 4 ml distilled water and lyophilized. 25 mg of the title compound (*S*)-CBA-Lys-Pec-  
5 Asp-Pro-Thr-leucinol was isolated as a fluffy, white solid (as the acetate salt, 86% yield). <sup>1</sup>H NMR (400MHz): consistent with proposed structure.

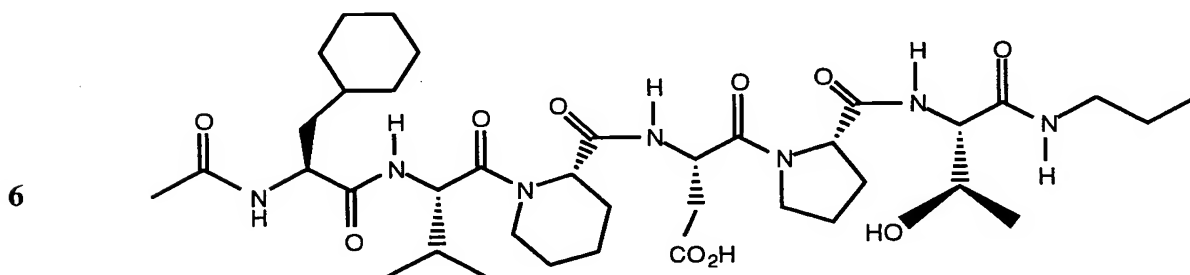
10 Compounds #5-#59, as shown in Table 1 below, may be synthesized by reference to the foregoing specific and general procedures for preparing compounds of Formula I.

Table I.

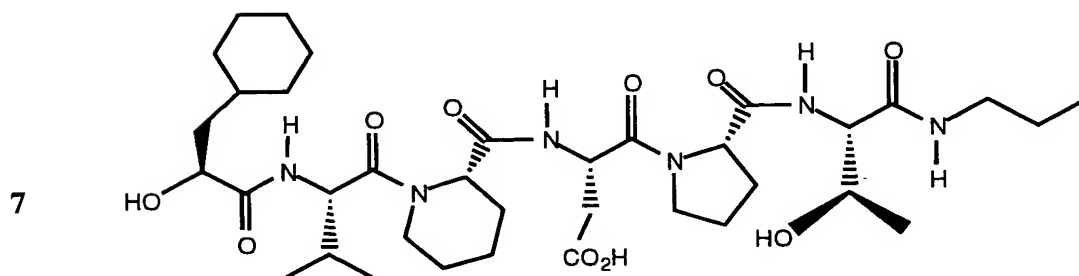
5	Example Compound No.	Structure
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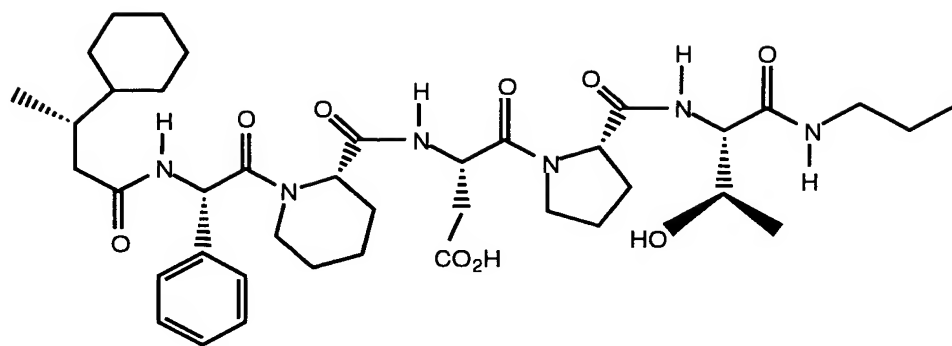
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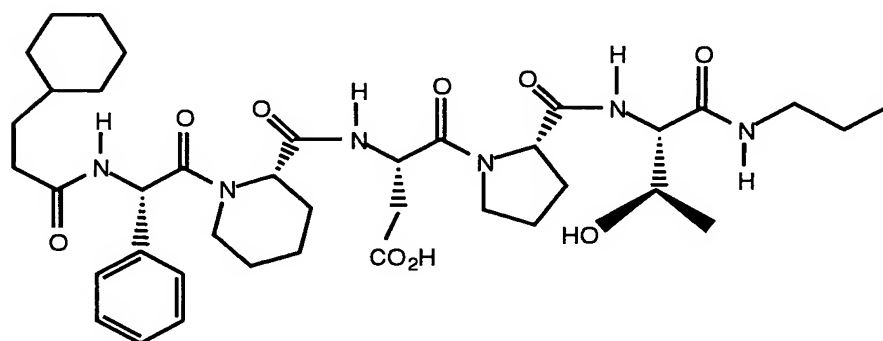


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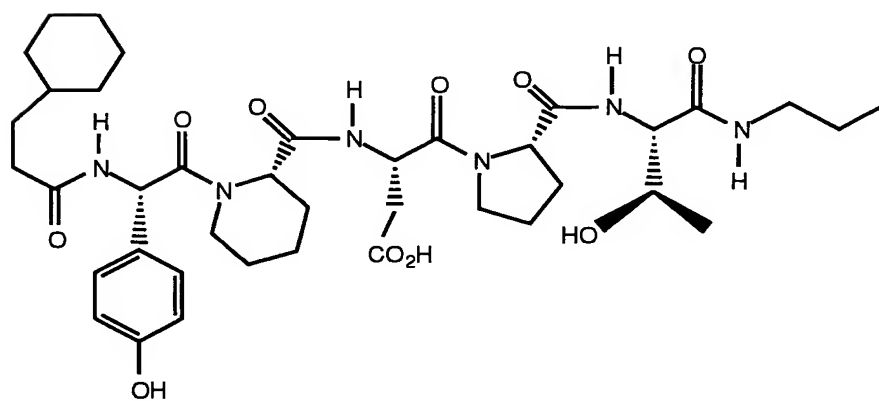


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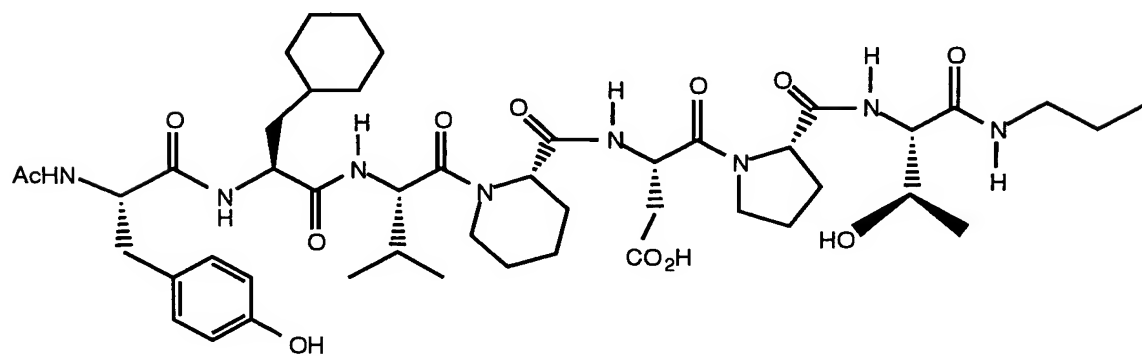


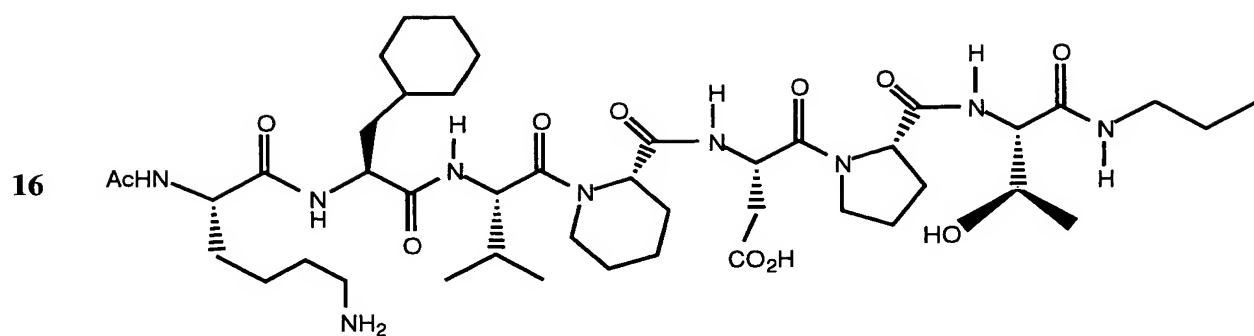
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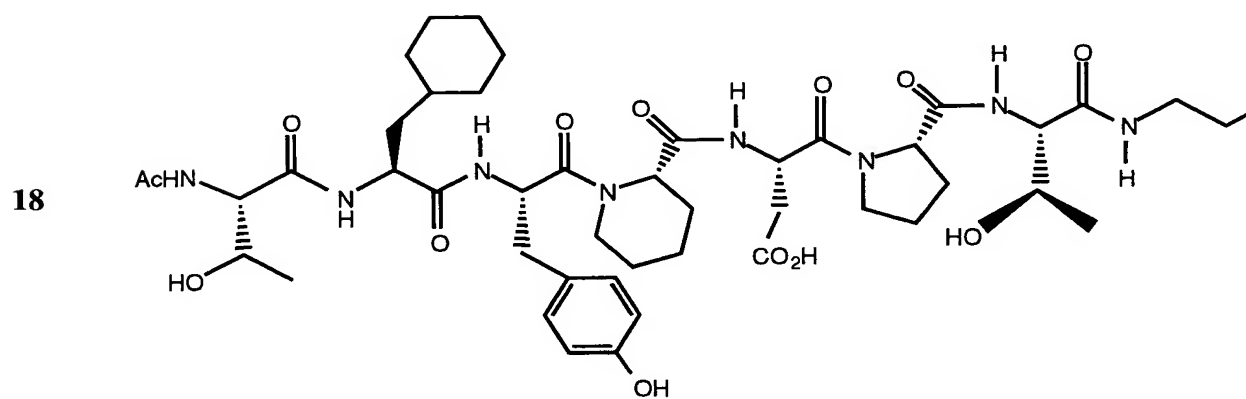
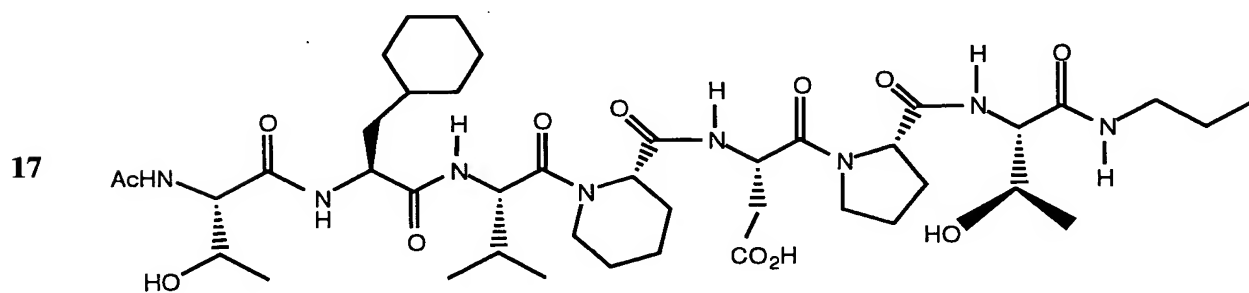


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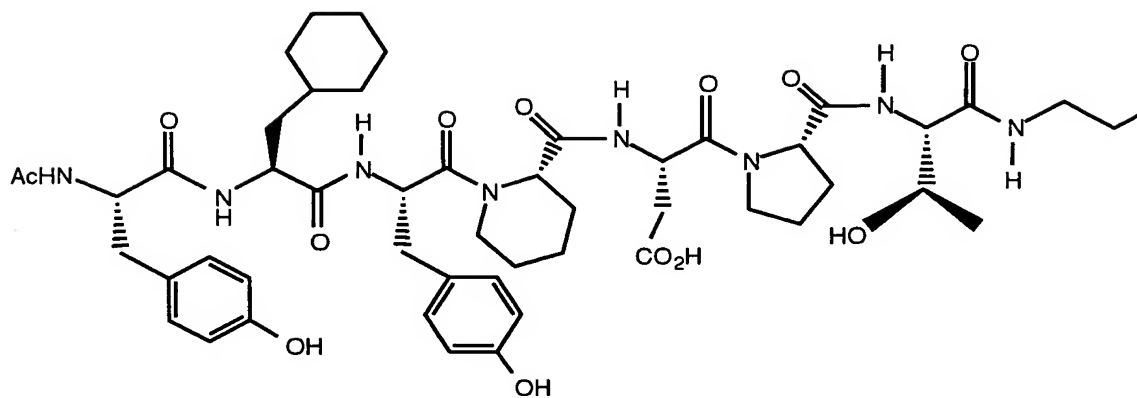
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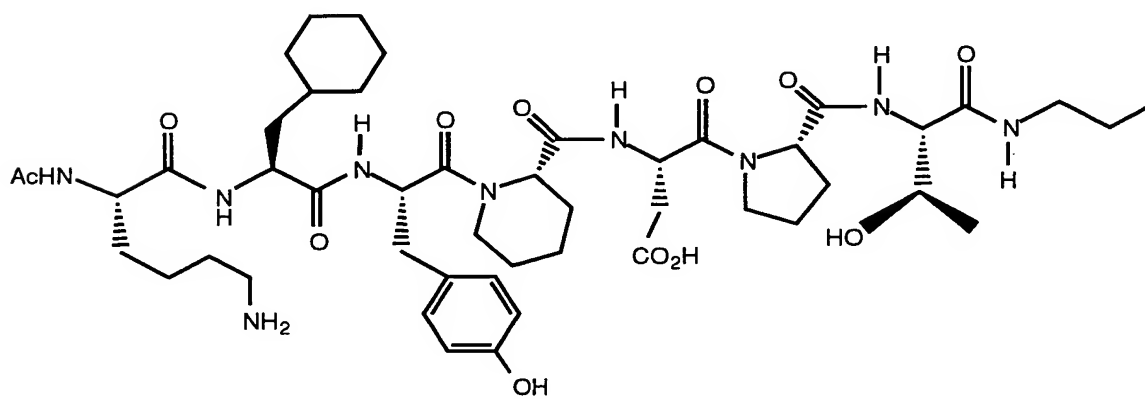
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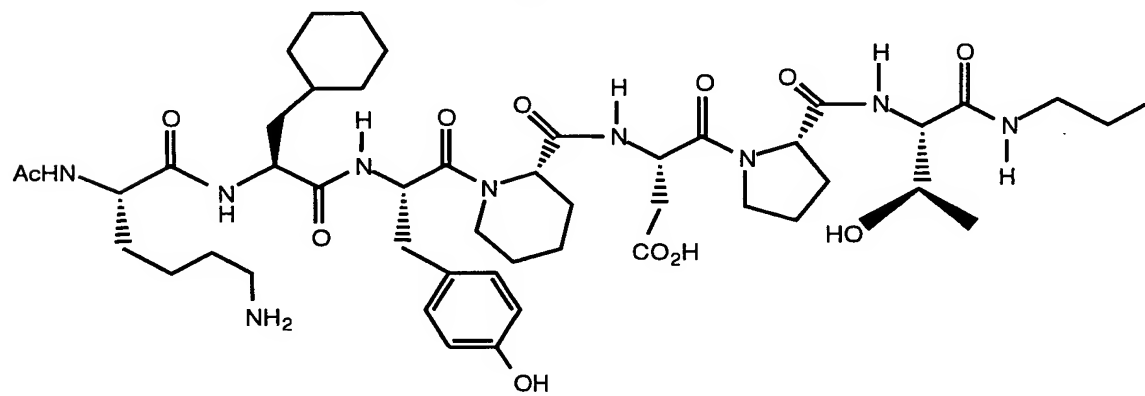
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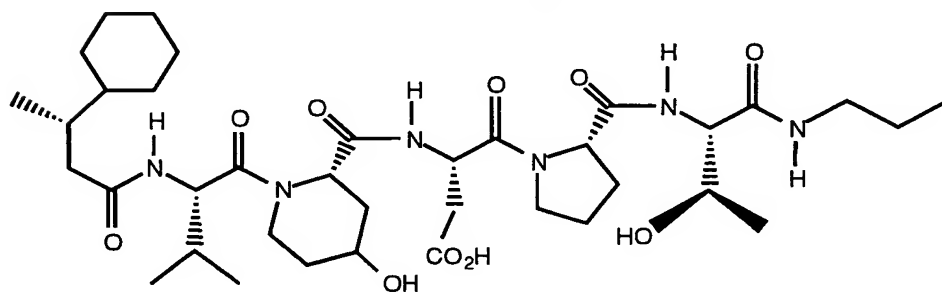


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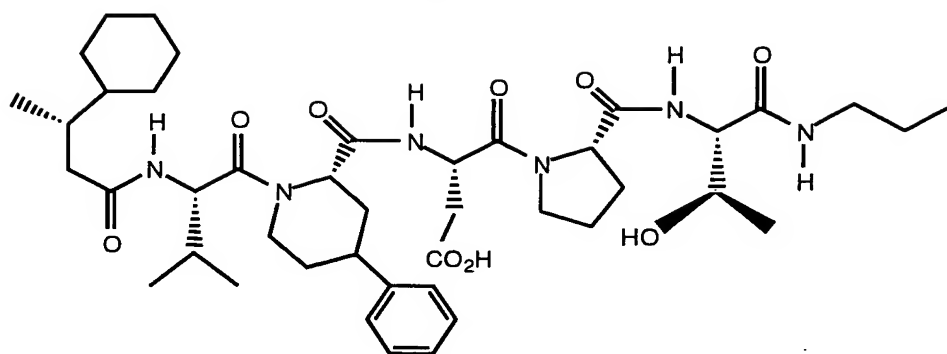
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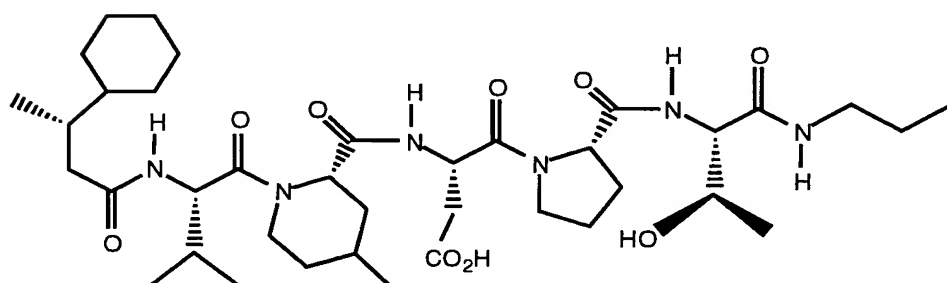


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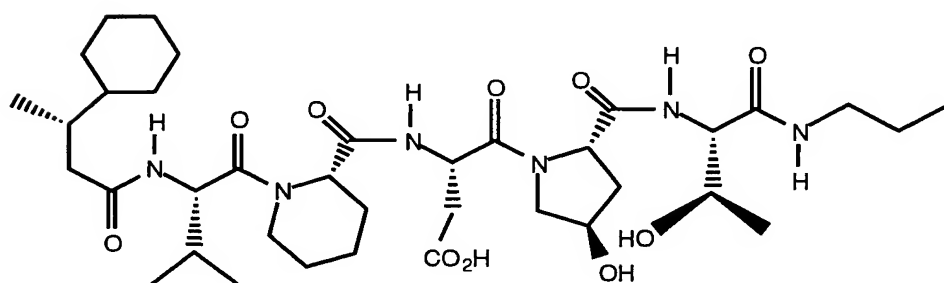


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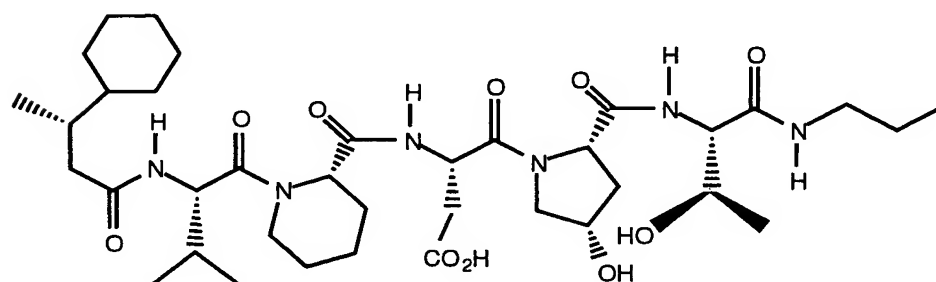


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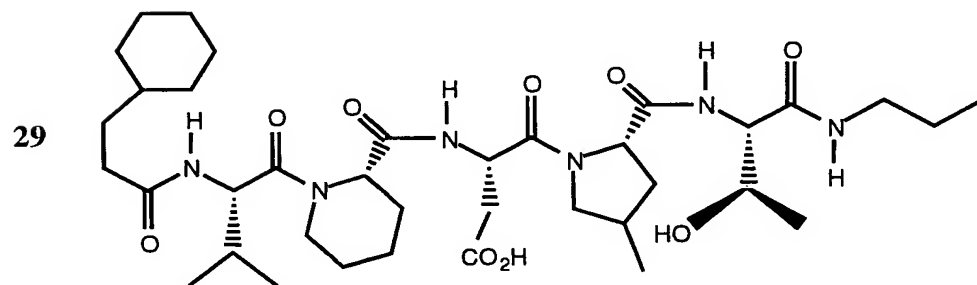
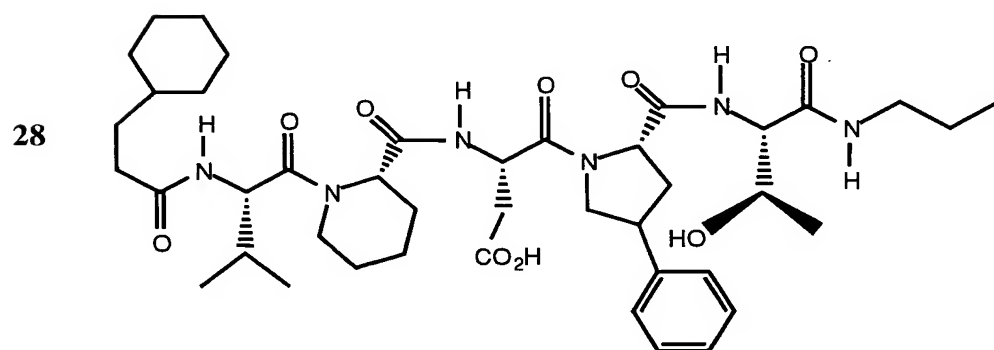
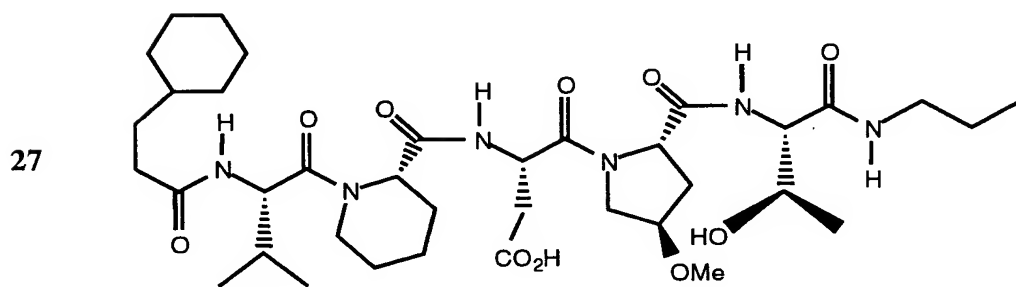
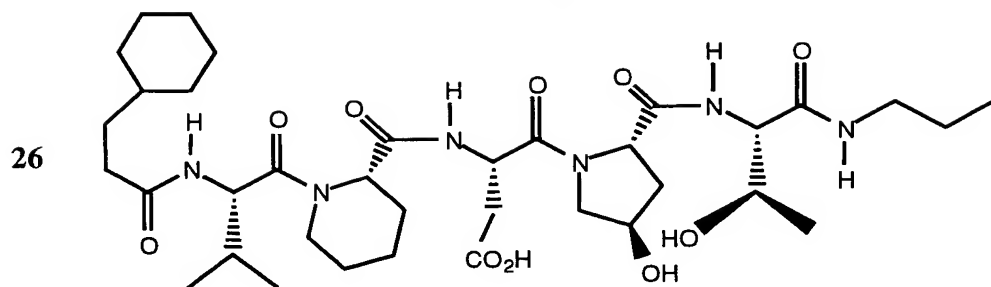


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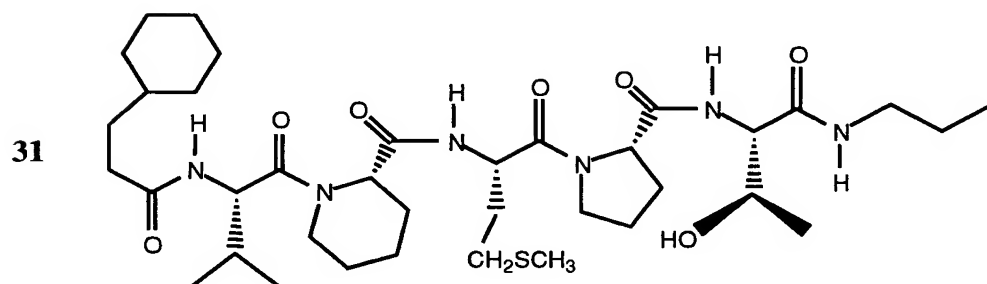
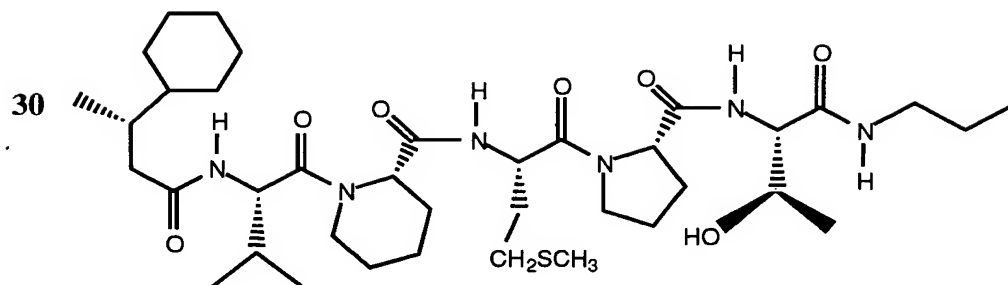


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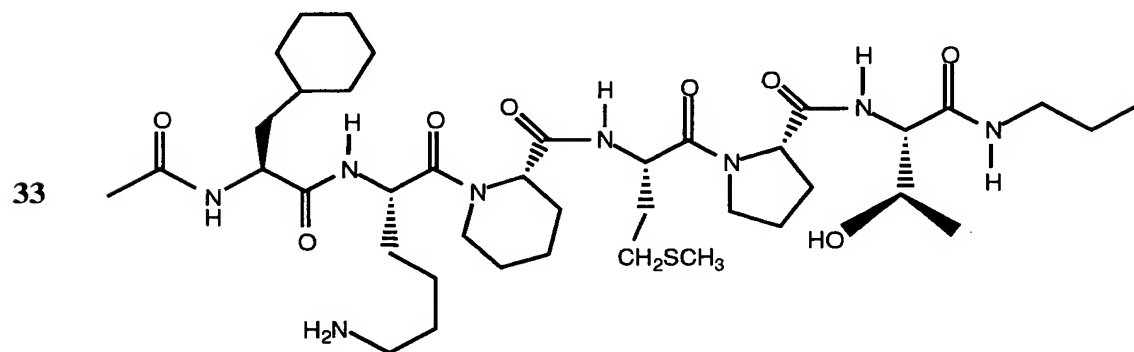
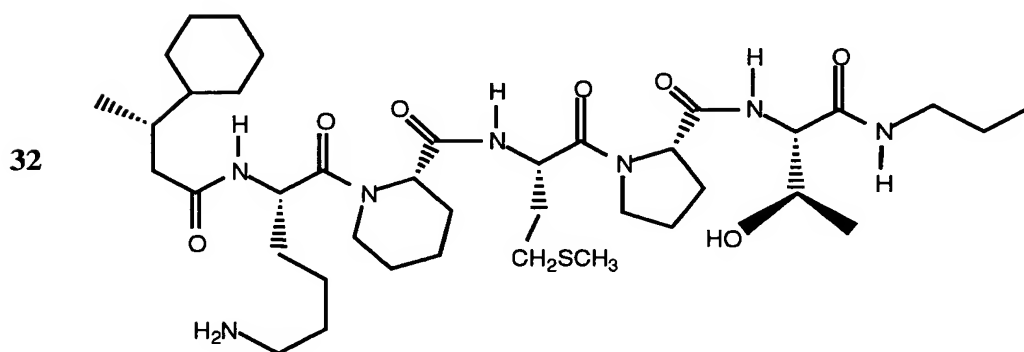
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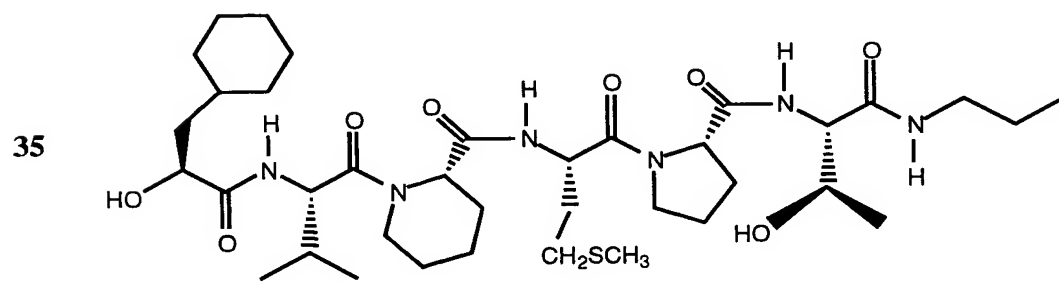
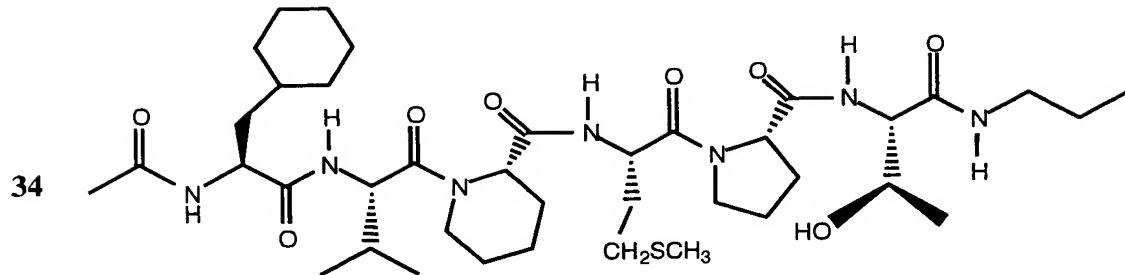


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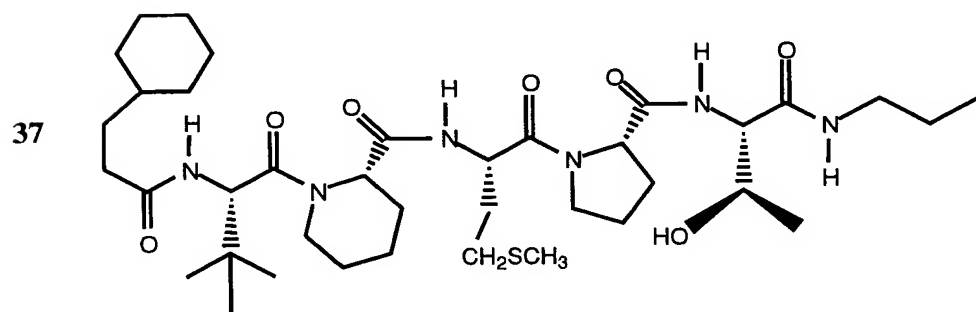
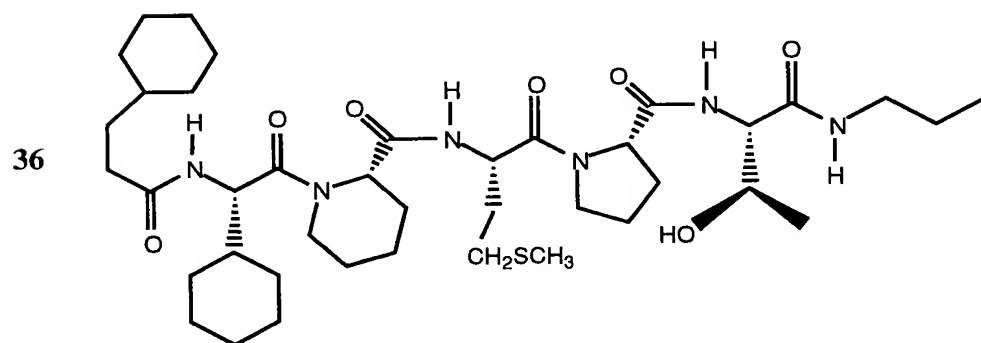


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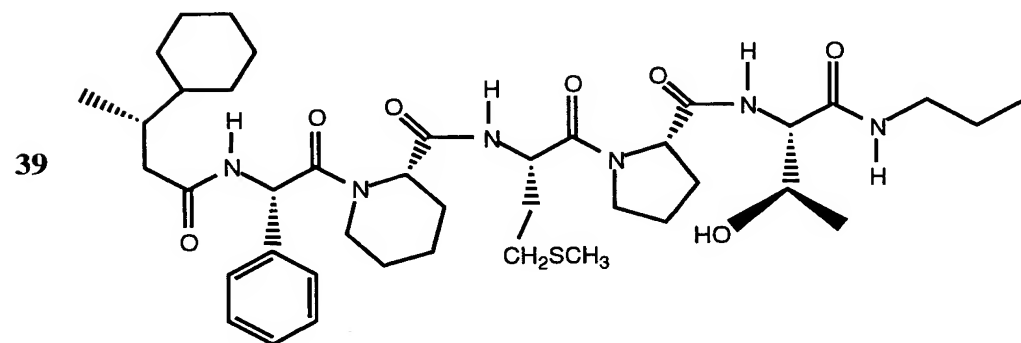
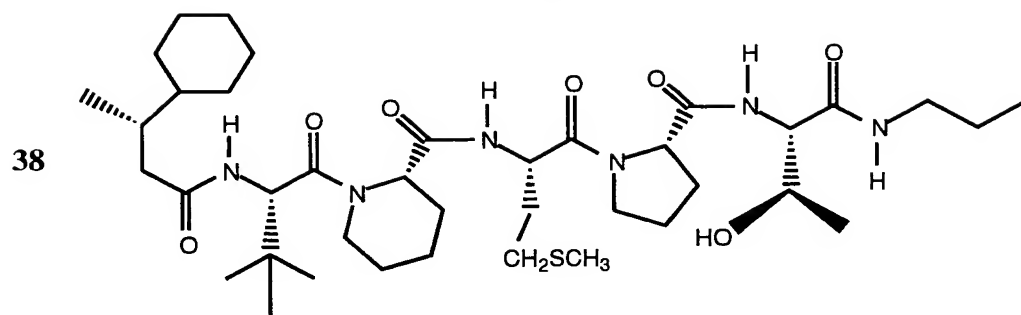


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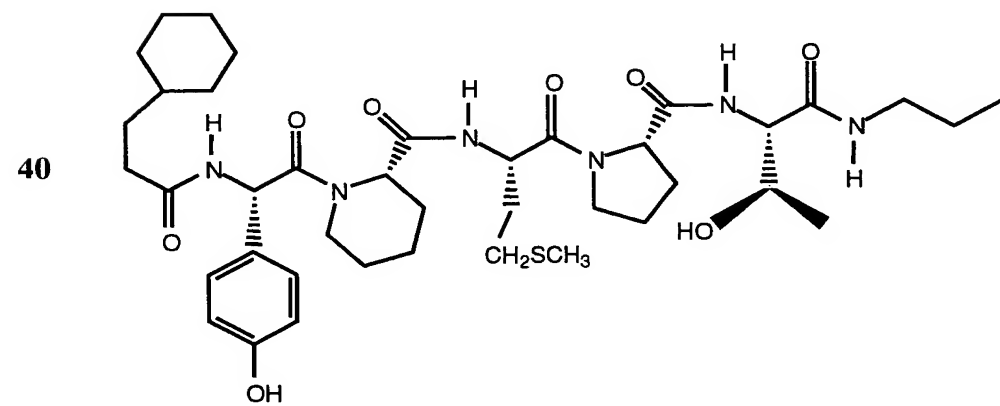


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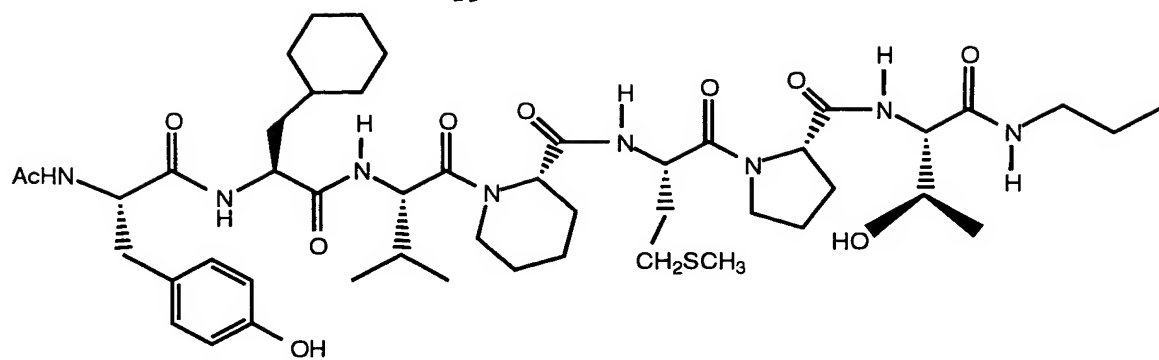


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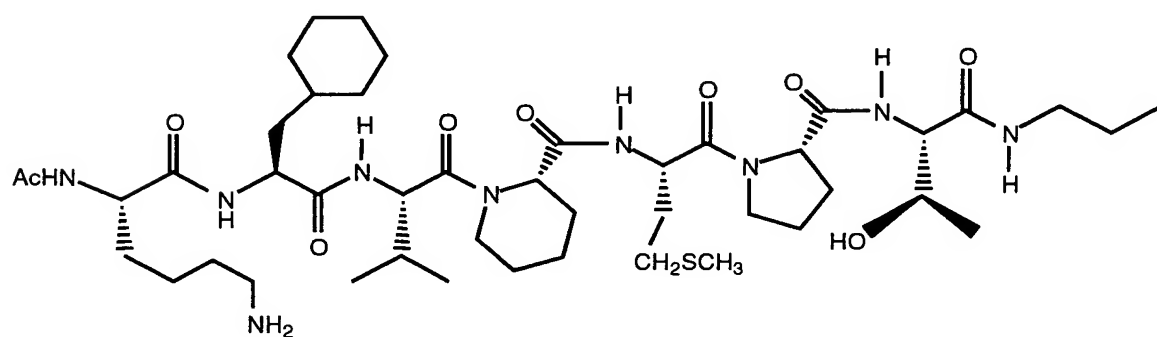


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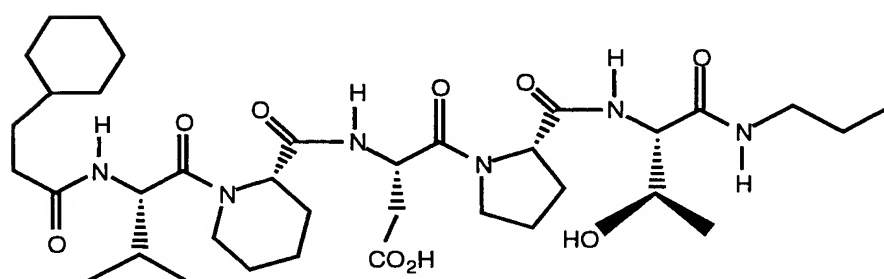


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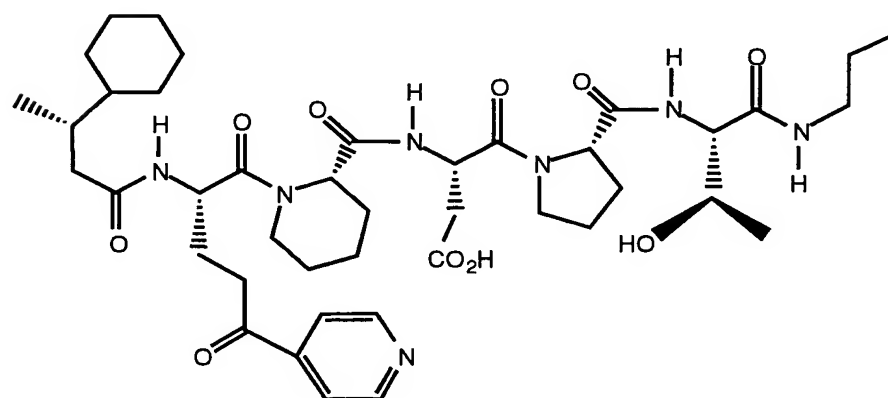
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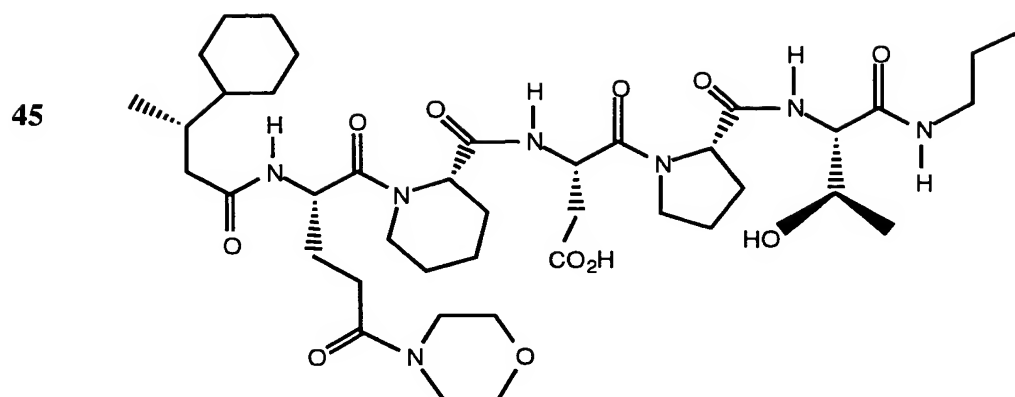
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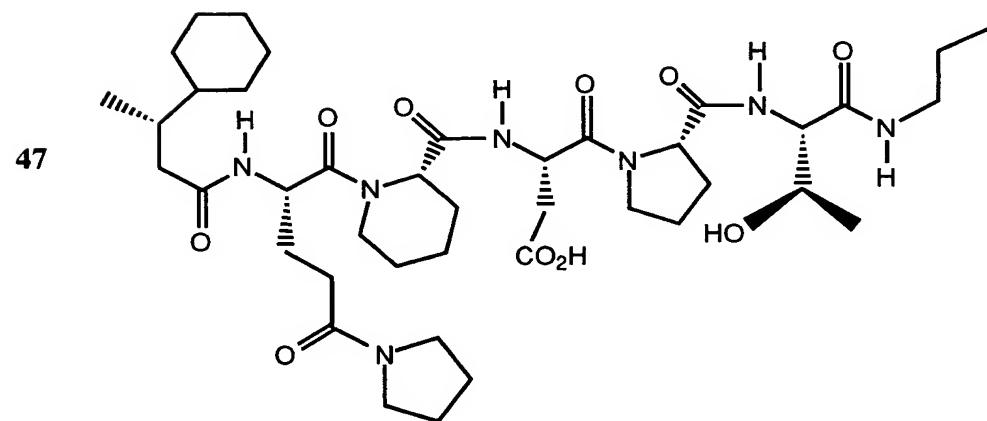
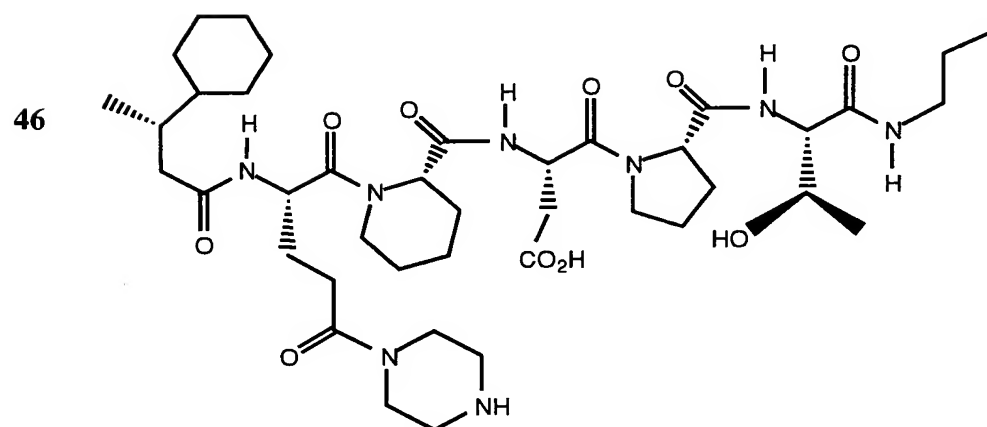
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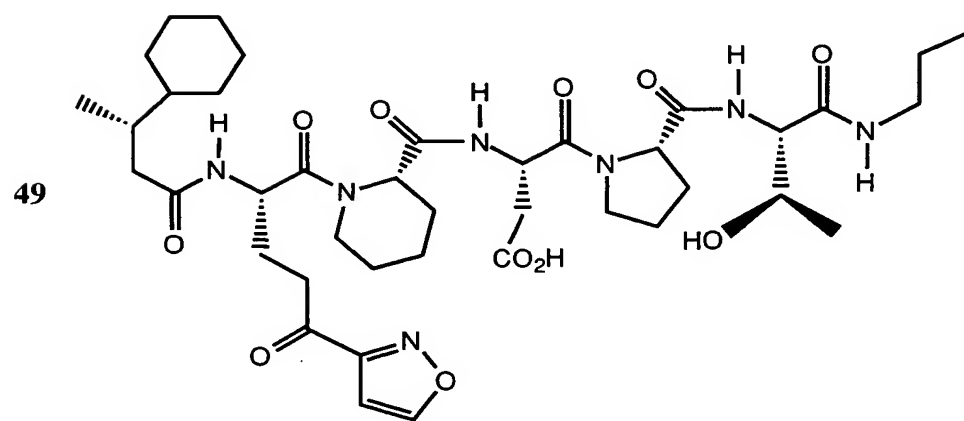
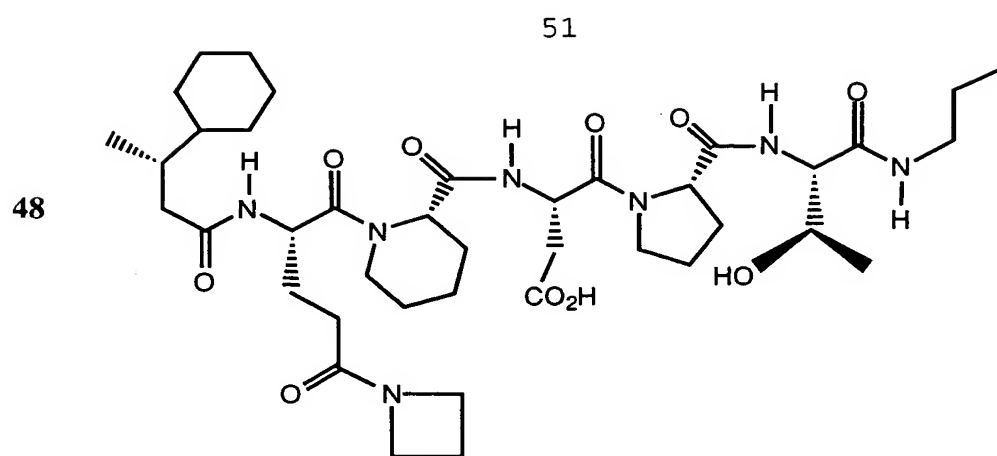
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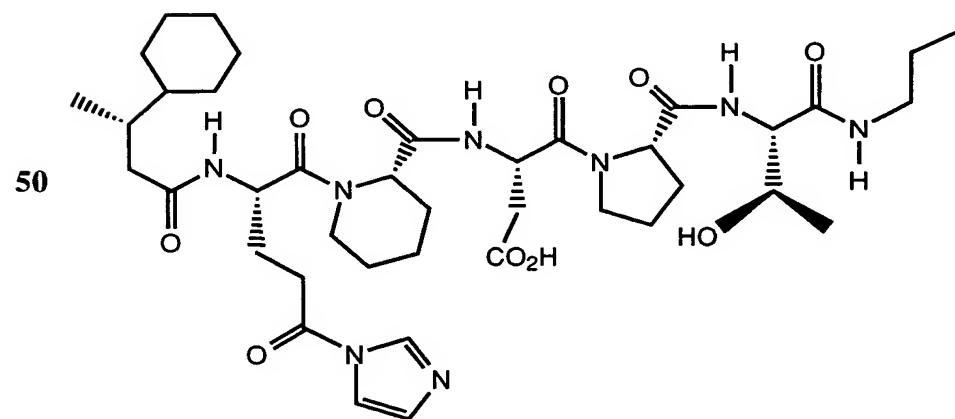


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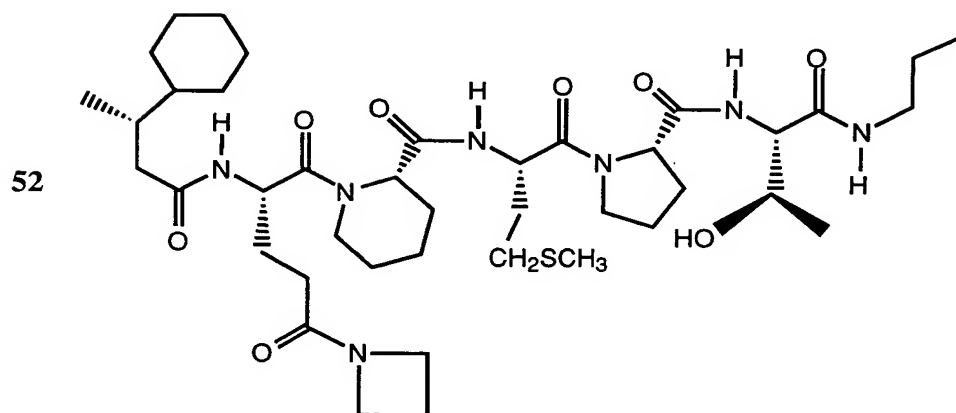
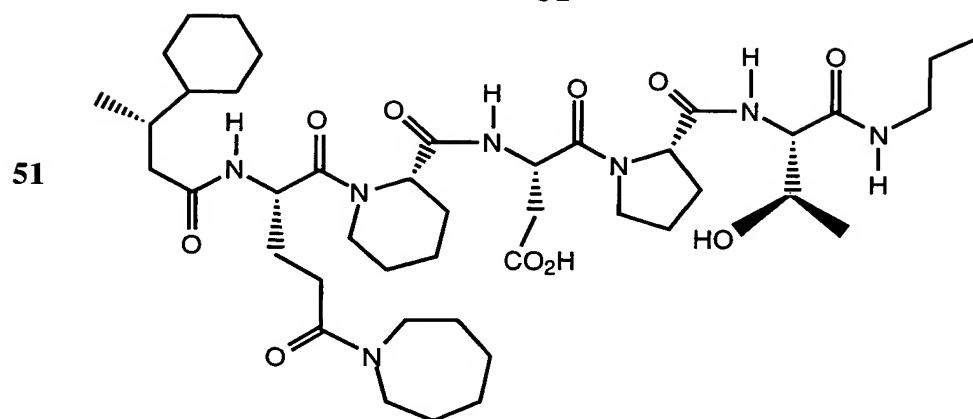




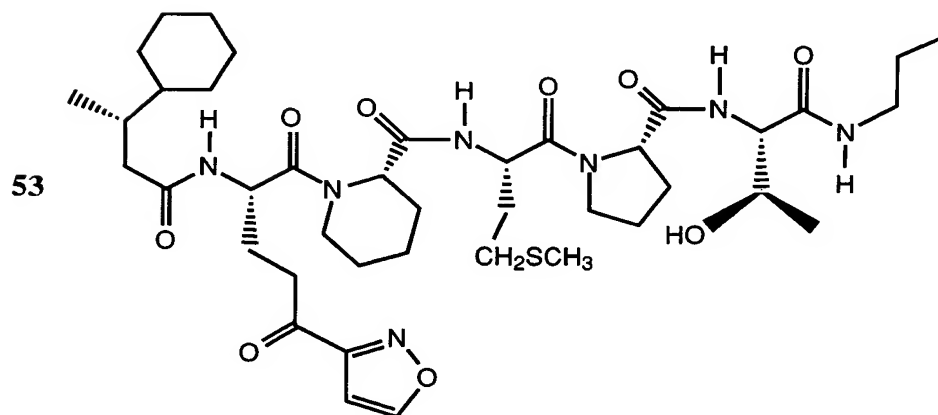
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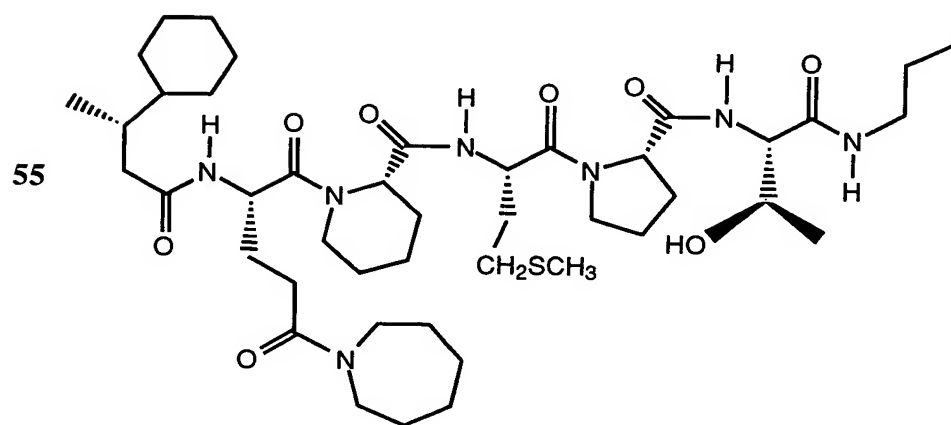
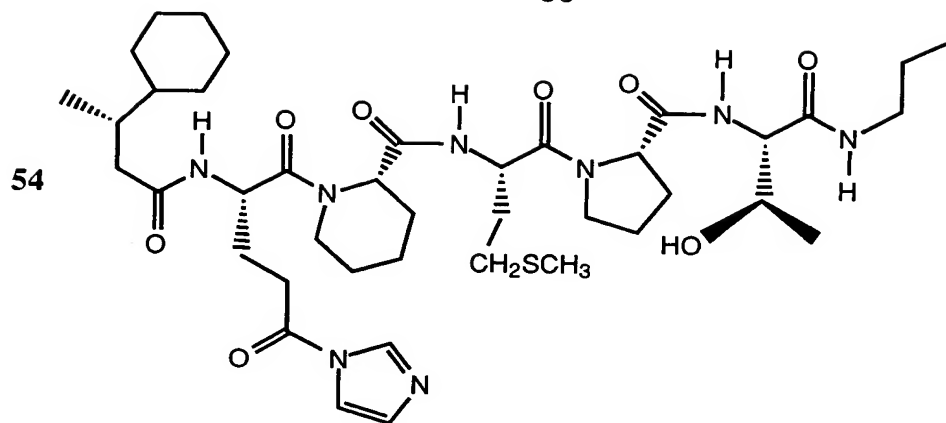
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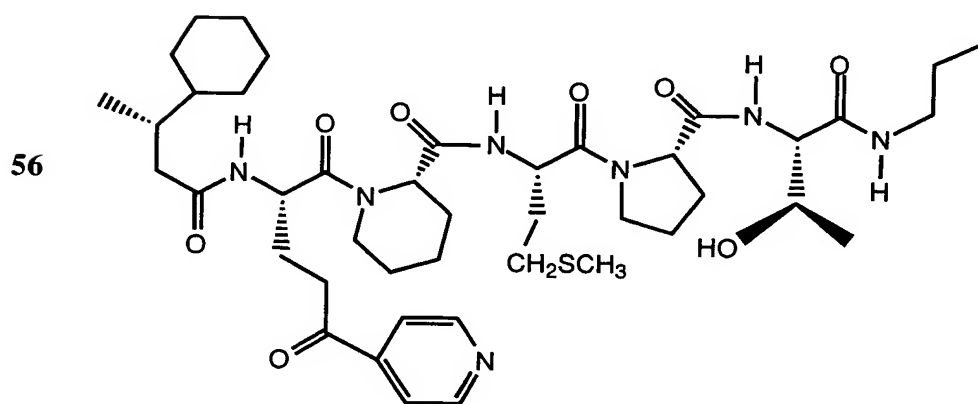
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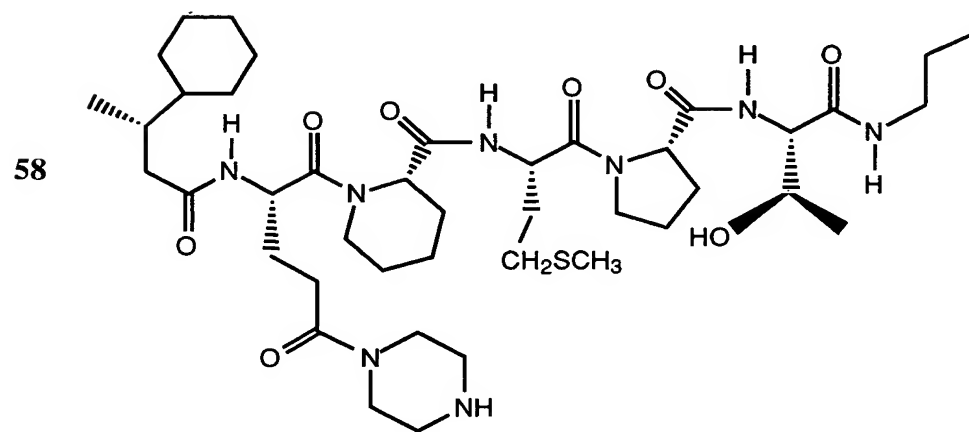
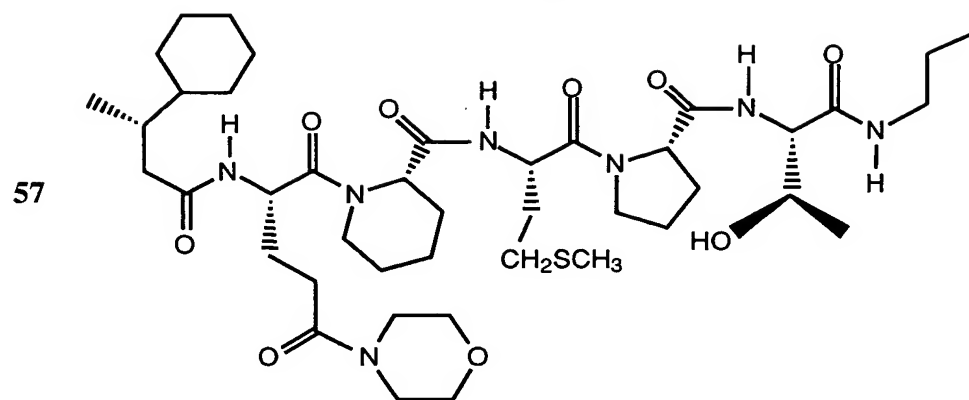
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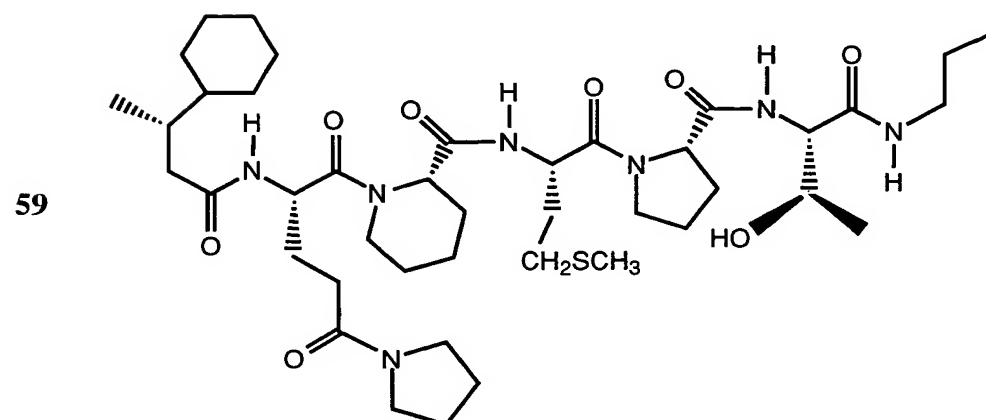
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**Biological Evaluation****Competitive Binding for DR4 (Competition Capture ELISA)**

5

DR( $\alpha\beta$ 1\*0401) molecules are affinity purified with L243 monoclonal antibody (hybridoma obtained from ATCC) using a modification of a published procedure (Gorga, J.C., et al., 1991. Res. Immunol. 142:401-407.).

10 Duplicate samples of immunoaffinity purified DRB1\*0401 molecules (0.5  $\mu$ g/well) are incubated overnight at 37 °C with 50 nM biotinylated ML37-49 peptide in the presence or absence of 1 nM to 100  $\mu$ M competitor peptides in 96-well polypropylene microtiter plates (Costar, Cambridge, MA). All dilutions are made in phosphate buffered  
15 saline, pH 5.5 containing 1% octagluconide (Boehringer Mannheim). LB3.1 antibody (obtained from J. Strominger, Cambridge, MA) is plated onto flat-bottom 96-well polystyrene microtiter plates (Falcon, Becton-Dickinson, Mountainview, CA) in 0.1 M carbonate coating buffer, pH  
20 9.6 overnight at 4°C. Antibody coated microtiter plates are blocked with 1% Carnation nonfat milk for 1 hr at room temperature. The plates are washed 3 times with PBS containing 0.05% Tween 20 (Biorad, Hercules, CA). The  
25 class II/peptide mixtures are added, incubated for 1 hr at 37°C, and washed as described above. Extravidin conjugated horseradish peroxidase (1:1000, Sigma Chemicals) is added to the wells and incubated for 30-60 min at 37°C. The plates are washed again, then incubated  
30 with o-phenylenediamine dihydrochloride and H<sub>2</sub>O<sub>2</sub> (Sigma Chemicals, St. Louis, MO) in 0.5 M phosphate-citrate buffer, pH 5.0. The absorbance of each well is read at 450 nm.

35 **Table II.** Competition Capture ELISA

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Example no.	56 IC50 (nM)
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5	1	30
	2	289
	3	259
	4	116

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### Inhibition of T cell proliferation Assay

Triplicate wells containing  $2 \times 10^4$  DR4 Dw4 positive antigen presenting cells (APC) are incubated in Excell 300 serum-free media containing 2 mM glutamine and 1% penicillin-streptomycin solution or in RPMI1640 containing 10% pooled human serum, 2 mM glutamine and 1% penicillin-streptomycin solution (R-10). The APC may be B-lymphoblastoid cells, L cells transfected with DR4Dw4, or peripheral blood lymphocytes. The APC are incubated under serum-containing or serum-free conditions with varying concentrations (0.01-500  $\mu$ M) of competitor peptides for 2-4 hr at 37 °C, 5% CO<sub>2</sub>. Suboptimal concentrations of HA307-319 peptide (0.01 to 1  $\mu$ M) or hemagglutinin protein are added to the wells at the same time as the competitor peptides (co-incubation) for 4 hr or for 2 hr following 2 hr of incubation with the competitor peptides (competitor pre-pulse). The plates are washed with the appropriate media three times to remove unbound peptides. The remainder of the assay is done in R-10.  $4 \times 10^4$  clonal human DR4 Dw4-restricted HA-specific T cells are added to the wells and incubated 3 days at 37°C, 5%CO<sub>2</sub>. PHA (5  $\mu$ g/ml) is added to some of the wells in the presense or absence of competitor peptides to determine if the peptides are cytotoxic. 3H-thymidine (0.5  $\mu$ Ci/well) is added to the cultures, incubated an additional 18 hr, and incorporation of radiolabel is determined using a  $\beta$ -scintillation counter.



Compounds of the invention are active in inhibiting the proliferation of T-cells in this assay.

Administration of the compounds of Formula I may be accomplished by administering a formulation containing an active compound by oral route, or by intravenous, intramuscular or subcutaneous injections. The formulation may be in the form of a bolus, or in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more pharmaceutically-acceptable carriers or diluents, or a binder such as gelatin or hydroxypropyl-methyl cellulose, together with one or more of a lubricant, preservative, surface-active or dispersing agent.

For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient. Examples of such dosage units are tablets or capsules. These may with advantage contain an amount of each active ingredient from about 1 to 250 mg, preferably from about 25 to 150 mg. A suitable daily dose for a mammal may vary widely depending on the condition of the patient and other factors. However, a dose of from about 0.1 to 3000 mg/kg body weight, particularly from about 1 to 100 mg/kg body weight, may be appropriate.

The active ingredients may also be administered by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable carrier. A suitable daily dose of each active component is from about 0.1 to 100 mg/kg body weight injected per day in multiple doses depending on the disease being treated. A preferred daily dose would be from about 1 to

30 mg/kg body weight. Compounds indicated for prophylactic therapy will preferably be administered in a daily dose generally in a range from about 0.1 mg to about 100 mg per kilogram of body weight per day. A more preferred dosage will be a range from about 1 mg to about 100 mg per kilogram of body weight. Most preferred is a dosage in a range from about 1 to about 50 mg per kilogram of body weight per day. A suitable dose can be administered, in multiple sub-doses per day. These sub-doses may be administered in unit dosage forms. Typically, a dose or sub-dose may contain from about 1 mg to about 100 mg of active compound per unit dosage form. A more preferred dosage will contain from about 2 mg to about 50 mg of active compound per unit dosage form. Most preferred is a dosage form containing from about 3 mg to about 25 mg of active compound per unit dose.

The dosage regimen for treating a disease condition with the combination therapy of this invention is selected in accordance with a variety of factors, including the type, age, weight, sex and medical condition of the patient, the severity of the disease, the route of administration, and the particular compound employed, and thus may vary widely.

For therapeutic purposes, the active component of this invention is ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered per os, the components may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets may contain a controlled-release formulation as may be provided in a dispersion of active

compound in hydroxypropylmethyl cellulose. Formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions  
5 may be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. The components may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed  
10 oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

15                   Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.